THE HORMONAL BASIS OF DECREASED GROWTH RATE IN BROILER CHICKENS EXPOSED TO HIGH ENVIRONMENTAL TEMPERATURES

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ABSTRACT

Broiler chickens and other meat animals grow very slowly at high ambient temperatures (such as those experienced in tropical and subtropical countries), and also exhibit a high mortality rate under these conditions. Experimental evidence suggests an interaction between thyroid hormones and growth hormone (GH) in the control of growth rate in heat stressed chickens. It is known that changes in the circulating concentrations of thyroid hormones are associated with altered growth rate in several species. Whilst thyroxine (T4) is chiefly secreted by the thyroid gland, in chickens the majority of the circulating tri-iodothyronine (T3) is produced in other tissues by 5'-monodeiodination, primarily in the liver. Regulation of hepatocyte deiodinase function is thus central in the control of thyroid hormone metabolism. The effects of the climatic environment upon the secretion and metabolism of thyroid and growth hormones (the thyrotrophic and somatotrophic axes) and their interactions may thus play a role in the alterations in growth rate associated with heat stress.

The present project was designed to investigate the responses of plasma thyroid and growth hormones to different degrees of heat stress (21, 25, 29, 32 and 35 °C in combination with various relative humidities) and to elucidate the mechanisms involved in the regulation of thyroid hormone economy under a range of environmental conditions.

The studies have confirmed that chronic heat stress depresses the circulating plasma concentrations of T4 and T3 in broiler chickens concomitant with reduced growth rate. The decreased plasma concentrations of T4 and T3 resulting from exposure to high ambient temperatures are not a consequence only of reduced food intake. The sensitivity of the peripheral 5'-monodeiodination of T4 to T3 to administration of exogenous thyrotrophin-releasing hormone (TRH) or chicken growth hormone (cGH) was decreased in chronically heat stressed broilers. In these

birds GH release in response to TRH injection was enhanced, and the apparent half-life of endogenous GH or exogenous cGH (chicken growth hormone) was increased. Heat stress thus inhibits GH stimulation of the *in vivo* conversion of T4 to T3 and this phenomenon may be associated with decreases in growth rate.

A further study using different "heat loads", rather than temperature alone was undertaken. This was achieved by simultaneously controlling and varying water vapour density or relative humidity (RH) values and dry bulb temperature. It was demonstrated that the hormonal response patterns are quite different in moderate and severe heat stress. The lowest rate of growth in severely heat stressed (at 29 °C & 85% RH) chickens is accompanied by large decreases in both T3 and T4 concentrations. In moderate heat stress (at 29 °C & 35% RH) T3 levels decline to a lesser degree, but T4 concentration is unaltered. The apparent stimulation of T3 production from T4 *in vivo* by growth hormone is reduced in proportion to heat load and increased body temperature. The results clearly demonstrated that thermal environments should be explicitly defined in terms of both temperatures and humidity when characterising the endocrine responses to "heat stress". The findings also emphasise the complexity of the interactions between the somatotrophic and thyrotrophic axes under such conditions. When assessing the effects of "hot" climates or heat stress upon endocrine function and growth in chickens, the environments should be characterised in terms of their potential influence upon thermal exchange and thermoregulation and not simply by measurement of dry bulb temperature.

It has been suggested by a number of field trials that broilers carrying the naked neck gene (Na) may be more heat stress resistant, and administration of vitamin C (ascorbic acid) or vitamin E (α-tocopherol) might improve growth rate of normal broilers under heat stress conditions. Further studies were, therefore, designed to examine the effects of these and other strategies upon thyroid function, and to examine the mechanisms involved.

It was demonstrated that heat stress produced less disturbances in thyroid hormone metabolisms in birds expressing the naked neck gene than in control broilers. Naked neck birds exhibit near "normal" T4 and T3 responses to TRH administration even under "moderate heat load". Several stressors, including excessive thermal loads, may cause tissue injury by the induction of free radical production and lipid peroxidation resulting in changes in the integrity of cell and organelle membranes. Vitamin E and ascorbic acid are recognised biological anti-oxidants and may thus be beneficial in reducing the detrimental oxidative effects of "stress". Supplementation of the diet with 500mg/kg ascorbic acid or vitamin E (500mg/kg) appeared to improve function of the somatotrophic/thyrotrophic axes during chronic heat stress without altering body temperatures. These results suggested that the antioxidant properties of dietary ascorbic acid or vitamin E might play an important role in maintaining 5'-monodeiodinase activity possibly by protection of hepatocyte membranes, and thus the function of GH-receptors mediating GH stimulation of hepatic T3 production.

The study provides important evidence of a possible endocrine basis for the derangement of growth rate induced by chronic heat stress in broiler chickens.

The results indicate that the roles and interactions of thyroid and growth hormones in the regulation of growth during heat stress are complex. Conflicting reports of the effects of heat stress upon thyroid function in poultry are probably a consequence of the range and duration of thermal loads employed, the use of birds from different strains, and their age, sex, diet and food intake responses.

DECLARATION

I hereby declare that this thesis has been composed by myself, and has not been submitted for any other degree, in Edinburgh or elsewhere. The work presented herein is my own, and all work of other authors is duly acknowledged. I also acknowledge all assistance given to me during the designing and execution of the experiments contained in this thesis and during the preparation of this thesis.

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PUBLICATIONS ARISING FROM THE THESIS

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Kan, P., Mitchell, M. A. and Carlisle, A. J., (1993). Effect of vitamin E on thyroid hormone production in heat stressed broiler chickens. **In:** *Proceedings of the Fourth European Symposium on Poultry Welfare,* Edinburgh, 18-21 September, 1993. (Edited by C.J. Savory & B.O. Hughes), Publisher: Potters Bar, Herts: Universities Federation for Animal Welfare. pp 295-297.

Mitchell, M. A., **Kan, P.** and Carlisle, A. J., (1993). The effects of differing heat loads upon plasma concentration of thyroid hormones and growth hormone in the domestic fowl. **In:** The 35th Annual Meeting of the *Climatic Physiology Group, Army Personnel Research Establishment, Farnborough, September, 1993*. (Edited by M. Tipton), Institute of Naval Medicine, Gosport, Hants PO12 2DL. Abstract S0937.

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DEDICATION

I dedicate this work to my father Prof. WeiBi Kan, my mother Prof. YuanZhi Hu, my Grandmother Ms. XiuYun Zeng, my aunt Ms. Yen King Kan and my son Mr. Koko Yuke Kan to whom it should act as a source of inspiration for hard work. I also dedicate it to my dear wife Ms. Hong Zhou whose commitment and support during the preparation of this work has been invaluable.

INTRODUCTION

Broiler chickens and other meat animals grow very slowly at high ambient temperatures, and also have a high mortality rate under these conditions. Artificial cooling systems, may improve growth rate in hot climates. Such systems, however, are expensive. Is it possible to promote growth of broiler chickens at high ambient temperature without cooling them? What are the mechanisms mediating reduced growth rate during heat stress? An understanding of the mechanisms may suggest strategies for the improvement of growth at high ambient temperatures without recourse to expensive ventilation or air conditioning systems. At high temperature, food intake decreases and birds will grow more slowly. The reason that birds eat less feed is to reduce their heat production (Klandorf, Sharp and MacLeod, 1981; Meltzer, 1987) and due to less energy requirement (MacLeod, 1992). The reason for reduced growth rate at high temperature, however, is not solely reduced feed intake. Fuller and Dale (1979) found that the body weight gain of heat stressed broilers was lower than that of thermoneutral pair-fed broilers examined between 4 to 7 weeks of age.

Integrated roles for hypothalamic thyrotropin-releasing hormone (TRH), pituitary thyrotropin (TSH) and growth hormone (GH), thyroid hormones and chicken hepatic 5'-deiodinase (5'-D) and GH regulation of the conversion of T4 into T3 in the control of growth rate in chickens, have been suggested. Growth requires the presence of GH and thyroid hormones (Buonomo and Baile, 1988). In birds, thyroid hormones are required for growth, particularly that of bone and muscle (King and King, 1973). Chemical thyroidectomy reduced the growth rate, a response which can be overcome by the administration of T4 (King and May, 1984). The effect of T4 and particularly T3 on growth in birds is critically determined by the dose administered or endogenously present in the bird (King and May, 1984). While low-to-intermediate levels of T3 and T4 are required for growth, high doses of T3 and to a lesser extent of T4 will depress growth. T3 stimulates growth in sex-linked dwarf

chicks with an isolated T3 deficiency (Bowen *et al.*, 1987). Administration of recombinant bovine somatotropin to 4-wk-old female broilers increased growth rate 6 to 7% after 1 wk of treatment (Buonomo and Baile, 1988). Unfortunately, similar attempts to increase growth in various breeds of normal broiler chickens, have met with limited success. It appears that there may be differences among various breeds in the mechanisms controlling growth rate in broiler chickens (Lam *et al.*, 1989; Kühn *et al.*, 1989). The fundamental processes involving thyroid hormones and growth hormone in control of growth rate in heat stressed broiler chickens require further study.

It is well established that nutrition has a profound modulatory effect on heat tolerance (Sykes and Fataftah, 1980; Männer, 1991), although the underlying mechanism is not well understood. Recent experimental evidence suggests that circulating metabolic hormones such as thyroid hormones and growth hormone, may play an important role in the control of heat tolerance in chronic heat stressed chickens (Bowen and Washburn, 1985; McCormick *et al.*, 1979; Sykes and Fataftah, 1986b; May, 1982). Study of these factors in chronic heat stressed chickens may facilitate a better understanding of the mechanisms controlling heat tolerance, and thus help to reliably improve growth performance in heat stressed broilers.

The major aim of this project was to characterise the thyroid and growth hormone responses in young broiler chickens chronically exposed to different quantified thermal loads. In these studies, attention was focused upon changes in the plasma concentrations of thyroxine, triiodothyronine and growth hormone as well as the hepatic 5'-monodeiodinase activities *in vivo* in broiler chickens during chronic heat stress. Circulating hormone concentrations have been examined and the control of peripheral T3 production has been addressed. The possible roles of endocrine adaptations in heat stress induced changes in growth have been considered. The effects of nutritional and genetic strategies for the improvement of growth rate during

heat stress upon thyroid hormone and growth hormone responses have also been investigated.

Chapter One

Literature review:

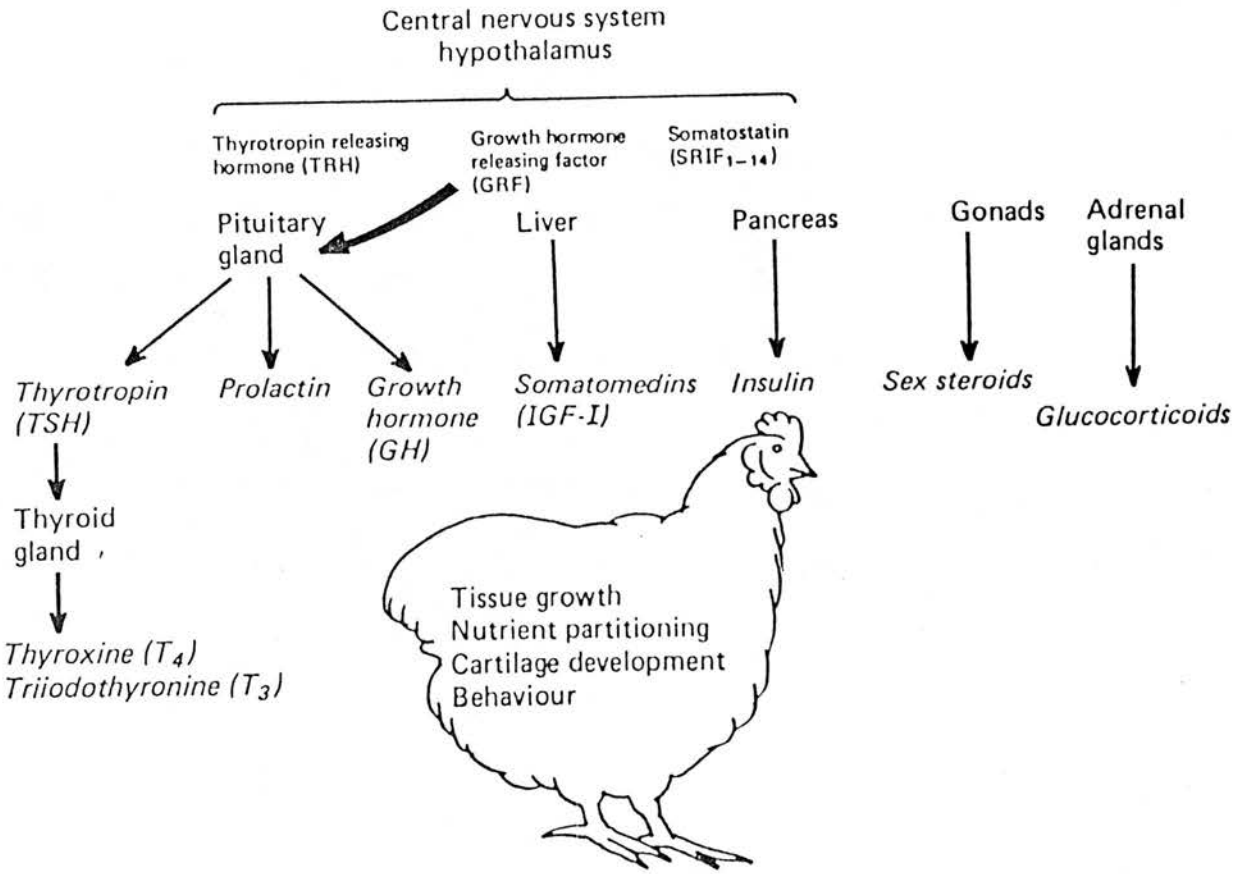
Physiological responses and adaptations to heat stress and the control of growth and metabolic heat production in birds

1.1. Endocrine control of growth in poultry

Growth is a dynamic and complex process that can be defined as biological synthesis. The development of an animal towards maturity is concerned with an increase in living substance and includes cell multiplication, cell enlargement and/or the incorporation of material from the environment (Johnson, 1989). Growth occurs in phases, during which the rate of change, the composition of the body mass and endocrine regulation vary with the stage of growth. It is important to know these growth stages for the animal species in question because their link with the endocrine system and other metabolic pathways may ultimately determine the ability to manipulate growth. Proportional growth is very rapid from hatching to 21-28 days of age and the relative rates of protein and fat deposition change dramatically and differ between the sexes.

Many hormones and growth factors are known to affect growth and development of poultry (Scanes *et al.*, 1984). There are summarized in Figure 1. One of the major organs involved is the pituitary gland, which is under the control of the hypothalamus and the central nervous system. However other organs of importance in growth regulation include the thyroid gland, liver, pancreas, gonads and the adrenal glands. The hypothalamus releases three peptides that regulate the secretion of growth hormone (GH) from the anterior pituitary gland, growth hormone releasing factor (GRF), thyrotrophin releasing hormone (TRH) and somatostatin (SRIF).

Figure 1. Hormones, growth factors and peptides which may be involved in growth regulation of poultry.



This figure summarises the hormones and growth factors that are known to affect growth and development of poultry. One of the major organs involved is the pituitary gland, which is under the control of the hypothalamus and the central nervous system. However other organs of importance in growth regulation include the thyroid gland, liver, pancreas, gonads and the adrenal glands. The hypothalamus releases three peptides that regulate the secretion of growth hormone (GH) from the anterior pituitary gland, growth hormone releasing factor (GRF), thyrotrophin releasing hormone (TRH) and somatostatin (SRIF). Adapted after Johnson (1989).

Growth hormone releasing factors with immunological similarities to those in mammalian species are present in the hypothalami of most lower vertebrates but have yet to be detected in birds (Harvey *et al.*, 1991b; Harvey, 1993b). Peptides with GH-releasing activity are present in the avian hypothalamus and presumably interact with the avian pituitary receptors stimulated by synthetic (mammalian) GRF preparations. The intracellular mechanisms involved in the induction of chicken GH release following GRF stimulation include an accumulation of cyclic adenosine 3',5'-monophosphate (cAMP) and the activation of protein kinase A (PKA) and protein kinase C (PKC) (Perez *et al.*, 1990). Calcium may normally participate in GRF-induced GH release (Perez *et al.*, 1989b; Goodman *et al.*, 1991) but secretion can occur independently of extracellular calcium entry into pituitary cells (Goodman *et al.*, 1991, 1992). The synergistic interaction between GRF and TRH in inducing GH release is also calcium-independent (Perez *et al.*, 1989b; Goodman *et al.*, 1992).

SRIF has been shown to inhibit basal GH release and GH release induced by the secretagogues GRF, TRH (Harvey *et al.*, 1991b). These actions appear to be mediated by a blockade of cAMP accumulation and impaired PKA activity (Donoghue and Scanes, 1991a).

The concentration of authentic TRH (pGlu-His-ProNH_2) in the chicken hypothalamus is far less (<10%) than that in the rat hypothalamus. The concentration of TRH in the hypothalamus is higher (by 2-3 fold) than the concentration in the pituitary gland of chickens (Harvey *et al.*, 1993c). Another tripeptide (pGlu-Glu-ProNH_2) with structural and immunological similarities to TRH is also found in these tissues. This peptide, in contrast with TRH, is present at higher concentration in pituitary glands than in the hypothalamus, suggesting a broiler pituitary site of synthesis. The pGlu-Glu-ProNH_2:TRH ratios in the broiler tissues are, however, much lower than those in mammalian species (Ashworth and Cockle, 1992; Ashworth *et al.*, 1991a,b). This peptide is undetectable in the hypothalamo-pituitary axis of

White Leghorn chickens (Harvey, 1993b).

It is well established that TRH is a potent GH-releasing factor in juvenile birds (Harvey *et al.*, 1991b; Harvey, 1990c) and that it stimulates the release of stored and newly-synthesised GH (Denver and Harvey, 1991). Thyrotrophin releasing hormone does not appear to stimulate GH synthesis *de novo*, in contrast with GRF (Denver and Harvey, 1991). The GH response of broiler fowl to TRH is less than in egg-laying White Leghorns (Harvey, 1993a). Although pGlu-Glu-ProNH_2 is similar to TRH, it has no direct effects on the release of GH from incubated broiler fowl pituitary glands (Harvey *et al.*, 1993c; Trudeau *et al.*, 1992). Unlike TRH, this peptide is also unable to downregulate TRH receptors on pituitary membranes.

Removal of the pituitary gland from poultry (hypophysectomy) results in a virtual cessation of bone growth. For example, in chickens hypophysectomized at 20-22 days old, body weight gain and bone growth rate at 49 days old was about 60% and 50% of controls respectively (Scanes *et al.*, 1986). These studies of growth provide direct evidence of the important role of a range of pituitary hormones in growth regulation,

To identify the more specific effects of individual hormones is more difficult. A 15% reduction in body weight gain over a 3 to 4 week period relative to normal controls was found after young chickens were treated with antibodies to growth hormone to deplete endogenous GH (Scanes *et al.*, 1977). Exogenous daily administration of mammalian GH has also been shown to significantly improve body weight gain and bone growth in hypophysectomized chickens, while mammalian prolactin increased only body weight gain (King and Scanes, 1986). Studies over the past several decades still have not resolved the question of the role of GH in growth of poultry.

Studies had shown TRH to be an effective GH secretagogue in poultry. Leung

et al. (1984a) have reported a growth-promoting effect of injected TRH in the chicken. Intermittent oral administration of TRH solution elevated GH concentrations in plasma (Burke and Vaughters, 1984). Continuous exposure of pituitary to TRH, results in refractoriness of insensitivity to stimulation. Although continuous exposure to TRH results in the loss of its GH releasing ability, basal GH was unaffected, suggesting other regulatory mechanisms were operating normally (Burke, 1987). Eventually even periodic exposure to TRH became ineffective in releasing GH. The response to TRH decreased and eventually was lost.

Perez *et al.* (1989a) showed that the effects of thyrotrophin-releasing hormone (TRH) and human pancreatic growth hormone-releasing factor (hpGRF) on growth hormone (GH) release are synergistic (greater than additive) in a primary culture of chicken adenohypophyseal cells. Cycloheximide (an inhibitor of protein synthesis) had no effect on basal GH release or hpGRF-induced GH release. However, cycloheximide abolished the synergy between TRH and hpGRF. Although neither TRH nor hpGRF alone stimulated GH production (intracellular GH plus GH release) during a 2-hr incubation period, in combination these secretagogues increased total GH. Perez *et al.* (1989a) suggest that GH release from the chicken somatotroph under conditions of TRH and hpGRF synergy requires protein synthesis.

The thyroid gland has been removed (thyroidectomy) to examine the direct influence of the thyroid hormones on growth. Thyroidectomy of chickens at 1-3 days old resulted in body weights that were only 56% of controls at 17 days old, with restoration of normal growth following exogenous administration of triiodothyronine (T3). Nuclear accumulation and proliferation within skeletal muscle fibres were also increased following T3 administration to thyroidectomized chickens, suggesting a key role for thyroid hormones in the regulation of muscle protein accretion in poultry (King and May, 1984).

The somatomedins, or insulin-like growth factors, consist of two related

peptides (IGF-I and IGF-II), and are secreted predominantly from the liver, primarily under the control of GH (Baxter, 1986). IGF-I is in the stimulation of cell division, cartilage growth and protein and fat synthesis (Hart, 1987) and has been referred to as the possible "ultimate endocrine link in the chain of hormones regulating cell growth" (Spencer, 1985). Plasma IGF-I concentration in growing poultry gradually increases with age for both layer and broiler strains. Hypophysectomy significantly depresses plasma IGF-I concentration, which increased towards normal by exogenous GH administration (Huybrechts *et al.*, 1985). Johnson (1989) have shown that plasma levels of IGF-I in broilers are relatively constant compared with GH, which has a pulsatile pattern of secretion. Plasma IGF-I levels are more variable in birds on *ad libitum* feed intake than during starvation, and there appears to be an inverse relationship between IGF-I and GH.

Growth hormone receptors are almost ubiquitous and the functions of most organs and tissues are influenced by GH action. Numerous factors in peripheral circulation may therefore feedback at hypothalamic or pituitary sites to regulate GH secretion. In birds, hormones produced by the liver, thyroid, adrenal, pancreas and gonads in response to GH stimulation may contribute to feedback inhibition (Harvey, 1993a). However, unlike mammalian species the hyperglycemic and lipolytic actions of GH (Scanes, 1992) appear to be unrelated to feedback regulation in birds, even though nutritional deprivation provokes GH release (Kühn *et al.*, 1991). The stimulatory action of GH on the avian immune system (Haddad and Mashaly, 1990; Marsh *et al.*, 1992) may, conversely, provide positive feedback, since cytokines have recently been shown to stimulate GH release in poultry (Buonomo, 1992).

Negative feedback in mammals is largely mediated by insulin-like growth factors (IGFs) produced in the liver. Hepatic IGFs are also GH-dependent in birds (O'Neill *et al.*, 1990). Whilst exogenous IGF-I can inhibit basal, TRH- and GRF-induced GH release in chickens (Perez *et al.*, 1985; Buonomo *et al.*, 1987). Birds are,

however, relatively insensitive to IGF-I (McGuinness and Cogburn, 1988). The physiological importance of IGF-I in the control of GH secretion in poultry is therefore uncertain, especially as endogenous GH concentrations may fluctuate independently of plasma IGF-I in normal birds (Lazarus and Scanes, 1988). The low circulating IGF concentrations in sex-linked dwarf birds has, however, been considered a causal factor in their constantly high concentrations of GH (Tixier-Boichard *et al.*, 1991). In birds a physiological role for thyroid hormones in the feedback inhibition of GH secretion has been proposed (Scanes *et al.*, 1990a), contrary to the stimulatory effects of these hormones on GH release in mammalian species.

1.2. Chemistry and physiology of poultry thyroid hormones

1.2.1. De-iodination

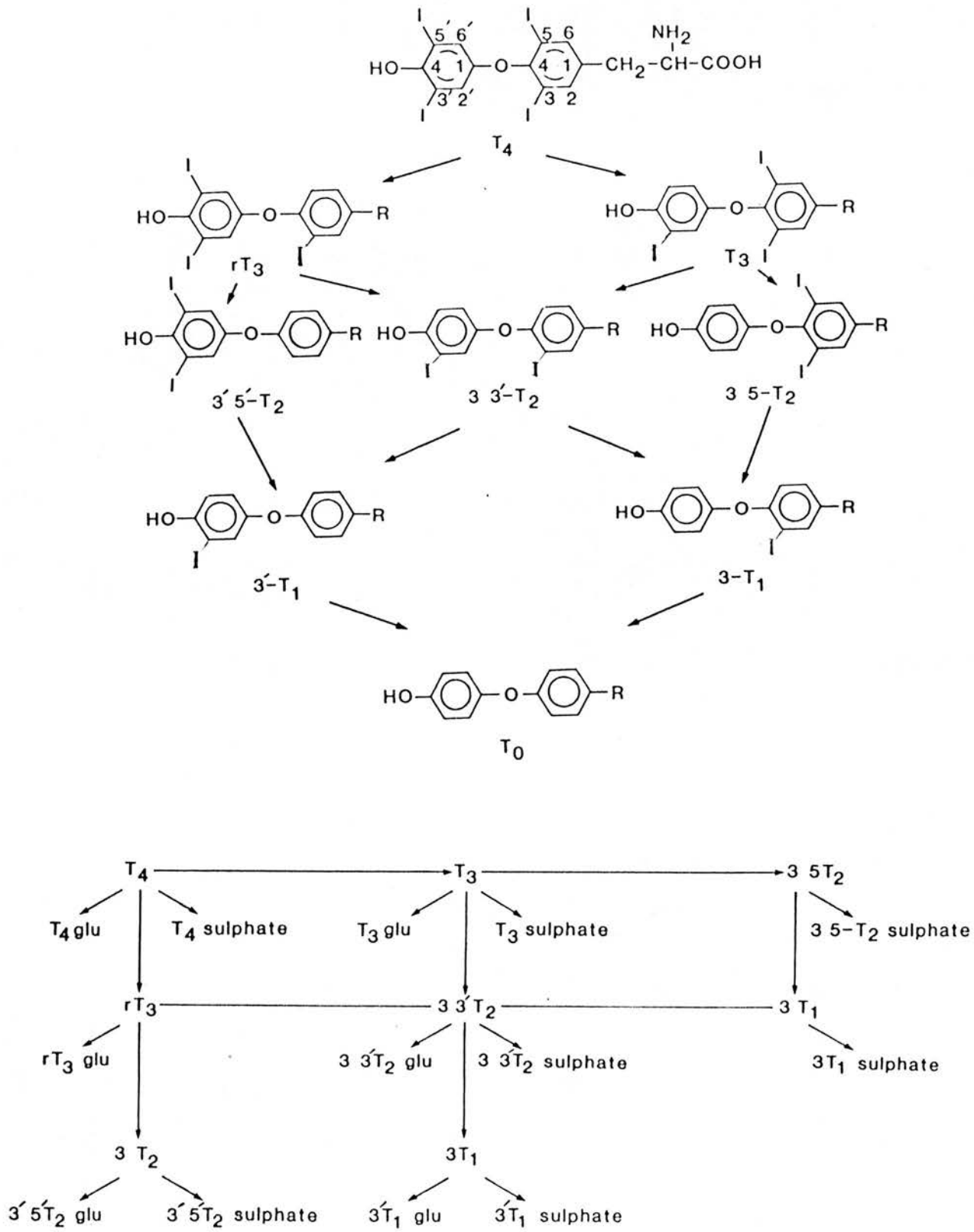
Thyroid hormones, which are synthesized in the thyroid gland play an important role in regulatory processes of tissue development and differentiation. Following the discovery of the 3,5,3'-triiodo-l-thyronine (T3) as the biologically active form of the thyroid hormone (Klandorf, Sharp and MacLeod, 1981; Guerrero and Reiter, 1992), it was proposed that T3 was primarily generated by extrathyroidal deiodination of thyroxine (3,5,3',5'-tetraiodo-l-thyronine; T4). Two general deiodination reactions have been identified based on their physiological and biochemical properties. One reaction leads to the formation of T3 by removal of a single iodine atom from the phenolic ring of T4 and is designated 5'-monodeiodination (5'-deiodination; 5'-D). T4 5'-deiodination is considered an activation pathway because it releases the biologically active thyroid hormone. One the other hand, removal of an iodine atom from the tyrosyl ring of thyroid hormones (T4 and T3) leads to the formation of inactive metabolites such as 3,3'5'-triiodothyronine (rT3) or 3,3'-diiodothyronine (3,3'-T2). The reaction is designated 5-deiodination and

considered an inactivating pathway. The relative activity of these 2 deiodination pathways determines the biological effect of thyroid hormones. There are different forms of the enzymes which monodeiodinate the phenolic and the tyrosyl rings of the T4 molecule (Visser, 1988; Leonard, 1990; Visser, 1990; Guerrero and Reiter, 1992). A wide variety of normal and pathophysiological conditions (development, diseases and nutritional state) are known to influence one or both of these deiodination pathways which show a coordinated regulation (Larsen *et al.*, 1981). A summary of the deiodinations of thyroid hormones and their metabolites are shown in Figure 2.

Thyroid hormone deiodination has been observed in various tissues, and at least three isoenzymes that catalyse the iodothyronine reactions have been identified (Kaplan, 1984; Hesch and Koehrle, 1986; Visser, 1988; Leonard, 1990; Visser, 1990). There are two isoenzymes stimulating 5'-deiodination. These two isoenzymes catalysing 5'-deiodination are referred to as type I and type II 5'-deiodinase. The third enzyme catalysing 5-deiodination is referred to as tyrosyl-ring deiodinase or type III 5-deiodinase. All three deiodinases may be membrane proteins associated with the tissue microsomal fractions, and for each of them simple thiols, such as dithiothreitol (DTT), act as cofactor (Kaplan, 1984; Hesch and Koehrle, 1986; Visser, 1988; Leonard, 1990; Visser, 1990). These three enzymes are defined by their different functional characteristics, as none of them has been purified. Thus, two major criteria serve to distinguish the three isoenzymes: (1) the reaction they catalyse (5- or 5'-deiodination) and (2), their susceptibility to inhibition by 6-propyl-2-thiouracil (PTU). Type I 5'-deiodinase isoenzyme is inhibited by PTU, while type II 5'-deiodinase and 5-deiodinase are not inhibited by the drug (Kaplan, 1984; Hesch and Koehrle, 1986; Visser, 1988; Leonard, 1990; Visser, 1990; Guerrero and Reiter, 1992).

High activities of the type I iodothyronine deiodinase (ID-I) are encountered especially in liver, kidney, and thyroid. It is generally believed that in normal subjects the deiodinases of liver and kidney have an important function in the production of

Figure 2. Monodeiodination and metabolism of thyroid hormones



This figure summarises deiodination and metabolism of thyroid hormones. Modified from Visser (1980) and Robbins and Rall (1979). (glu = glucuronide)

plasma T3 and the clearance of plasma rT3 (Visser *et al.*, 1988).

Compared to the type I isoenzyme, type II has a more limited distribution. The type II iodothyronine deiodinase (ID-II) is present in the central nervous system (CNS), brain, anterior pituitary gland, brown adipose tissue, placenta, pineal gland, Harderian gland and human epidermal keratinocytes; it deiodinates only the outer ring, showing a preference for T4 over rT3 (Kaplan, 1984; Hesch and Koehrle, 1986; Visser, 1988; Leonard, 1990; Visser, 1990). The enzyme is important for the local supply of T3 by T4 in these tissues, and it may contribute significantly to the peripheral production of plasma T3 in hypothyroid subjects (Silva *et al.*, 1984). In the brain, type II 5'-deiodinase (5'D-II) activity is primarily found in neurones, although glial cells can be induced to express the enzyme by drugs that increase intracellular levels of cyclic AMP (Leonard, 1990). In the pituitary gland, lactotrophs and somatotrophs have the highest enzyme activity while thyrotrophs have the lowest activity (Leonard, 1990). In other tissues, the specific cellular location of the enzyme remains unidentified.

The type III iodothyronine deiodinase (ID-III) is found especially in brain, skin, and placenta; it catalyses the deiodination specifically of the inner ring, with some preference for T3 over T4 (Kaplan, 1984; Hesch and Koehrle, 1986; Visser, 1988; Leonard, 1990; Visser, 1990). In the central nervous system the activity of this enzyme appears to be coordinated with that of ID-II to secure optimal regulation of intracellular T3 levels, and the type III deiodinase in brain and skin perhaps also function in the clearance of circulating T3 and the production of plasma rT3 (Kaplan, 1984; Hesch and Koehrle, 1986; Visser, 1988; Leonard, 1990; Visser, 1990).

It is known that ID-III is present in chick embryo and posthatch chick liver (Galton and Hiebert, 1987; Kühn *et al.*, 1992; Darras *et al.*, 1992a,b, 1993), and monkey hepatocarcinoma cell lines (Sato and Robbins, 1980). It has been shown, however, that adult rat hepatocytes in addition to ID-I also contain a type III-like

deiodinase (Sato and Robbins, 1981; Eelkman *et al.*, 1989). This fact was concluded not only from observations of a PTU-resistant conversion of T3 to 3,3'-T2 and of T4 to rT3 in primary cultures of rat hepatocytes but also from studies using rat liver microsomes (Eelkman *et al.*, 1989). Thus, production of 3,3'-T2 from T3 by microsomes and DTT is only partially blocked by complete inactivation of ID-I by rT3 plus PTU. The residual, PTU/rT3-resistant enzyme activity shows relatively high "affinities" for T3 and T4 and a high DTT requirement, characteristic features of ID-III (Eelkman *et al.*, 1989).

1.2.2. The transportation of thyroid hormones and their metabolites

Thyroid hormones, which are synthesised and secreted by the thyroid gland and are bound to serum transport proteins, play an important role in regulatory processes of tissue development and differentiation. Thyroxine is delivered to its target receptors in the nucleus where the transcription process is regulated. The more potent hormone, T3, which mediates all essential metabolic and developmental effects, is produced primarily in peripheral tissues by iodothyronine deiodinase (Larsen *et al.*, 1981).

The absence of thyroxine-binding α-globulin (TBG), which was identified in mammals, in avian species has been confirmed by many researchers. Both T3 and T4 are bound to serum albumin, and the binding affinity of albumin for T3 and T4 is the same (Tata and Shellabarger, 1959; Tritsch and Tritsch, 1965; Refetoff *et al.*, 1970; Grandhi *et al.*, 1975). Binding of T4 to prealbumin has also been reported in the fowl although T3 appeared to be bound only to albumin (Refetoff *et al.*, 1970). Heninger and Newcomer (1964) noted that T4 in chicken plasma is bound to the protein-binding sites with greater affinity than is T3. A α-globulin similar to TBG and representing the major T4 transport protein has been demonstrated in the budgerigar (Robiller *et al.*, 1975). Mitchell and Stiles (1985) suggested that previous conflicting results in birds

were erroneous due to the choice of inappropriate analysis techniques. A fraction with binding properties corresponding to an inter-α-globulin has been isolated from chicken plasma (Bhat and Cama, 1978,1979). In immature chickens Davison *et al.* (1978) observed that the major binding protein for T4 and T3 was serum albumin. Some T4 but little T3 was bound to prealbumin-2, whereas a third of the T3 was bound to an α-globulin but less than 10% T4 was found in association with this protein. In adult quail McNabb and Hughes (1983) reported a thyroxine-binding profile similar to that of immature chickens but half the T3 was bound to α-globulin and the remainder equally divided between albumin and γ-globulin.

In addition to the above thyroid hormone-binding proteins, three plasma components possibly involved in T3 and T4 transport have also been identified in chicken plasma using agarose (A 1.5 m) gel filtration at pH 7.4 (Mitchell and Stiles, 1985). Mitchell and Stiles (1985) observed that in mature hen, though not male and immature female birds, 11% of T3 is bound to very-low-density lipoprotein and low-density lipoprotein (VLDL/LDL). These could mediate transport of the hormone into the egg. Similarly vitellogenin appears to bind small quantities of both T3 and T4. A low-molecular-mass protein (9×10^3 D) which preferentially binds thyroxine in substantial amounts (20-30%), was also found in plasma from adult male and female and immature female birds (Mitchell and Stiles, 1985). VLDL/LDL are found in large amounts in laying hen plasma but not in male or immature female chicken plasma. These proteins are induced by oestrogens and are required for egg yolk synthesis (Board and Hornsey, 1978). Mitchell and Stiles (1985) observed that binding of T3 and T4 to VLDL/LDL in only the adult females and little or no T3 and T4 was eluted in this region in males or young females. The binding appeared to be selective for T3; a much higher percentage of this hormone than of T4. The 11 % of T3 bound to lipoprotein would represent a total of over 12 ng in a 1.5 kg bird (Mitchell and Stiles, 1985).

Another oestrogen-dependent plasma protein required for egg formation (Butler, 1983) is vitellogenin. Small amounts of both T3 and T4 appeared to be bound to this molecule in the adult females but not in males or juvenile females. Confirmation of the thyroid hormone binding by the two classes of egg yolk precursors in the laying hen was obtained from the lipoprotein depletion experiments. The procedure employed has been shown to selectively remove VLDL/LDL and vitellogenin from laying-hen plasma (Griffin and Mitchell, 1984). Further confirmation of the nature of the thyroid hormone-binding components was obtained from the experiments upon cockerels treated with either laying-hen plasma or oestradiol by Mitchell and Stiles (1985). Selective precipitation of the lipoproteins and vitellogenin from laying-hen plasma produces a binding profile identical to that observed in male and juvenile female birds. Treatment of adult male birds with oestrogen induces a binding profile for T3 similar to that observed in adult females (Mitchell and Stiles, 1985).

The third newly recognised thyroid hormone binding component is apparently thyroxine specific protein and has a low molecular weight (9×10^3 D), which preferentially binds thyroxine in substantial amounts (20-30%) was found in plasma from adult male and female and immature female birds (Mitchell and Stiles, 1985).

1.2.3. The potency of T3 and T4 in mechanism of action

The relative potencies of tri-iodothyronine (T3) and thyroxine (T4) in birds is difficult to determine because the thyroid hormone predominantly released from the thyroid gland is T4, although a little of T3 is also produced from the thyroid gland the majority of the circulating T3 is produced from other tissues by 5'-monodeiodination (5'-D), primarily in the liver (Borges *et al.*, 1980). Peripheral conversion of T4 to T3 through 5'-monodeiodination has been demonstrated in the chicken (Sharp and Klandorf, 1985). Initial studies in domestic birds showed that T3 constituted approximately 40% and T4 approximately 60% of the levels of circulating thyroid hormones (Wentworth and Mellen, 1961). In man, thyroxine-binding globulin is the

primary carrier of T4 and, to a lesser extent, T3. The thyroid gland is a structure which traps and stores iodide from the blood. Inside the gland iodide is coupled to a large protein and stored. The iodoamino acids are used in the synthesis of thyroid hormones, the most important of which are T4 and T3. For most organisms the supply of iodide is both scarce and highly discontinuous and so the ability of the thyroid cell to draw upon such a reserve when the intake of iodide is low represents an important homeostatic mechanism. The biochemistry of the thyroid gland is thus geared to both retain and to make the most efficient use of iodine.

T3 and T4 have different relative potencies when different functions are evaluated. T3 is more active than T4 in stimulating intracellular accumulation of certain neutral amino acids by embryonic chick bones (Adamson, 1970). There is evidence in chickens that T3 rather than T4 is the metabolically active thyroid hormone (Klandorf *et al.*, 1981). In respect to stimulation of metabolic heat production (MHP) test in which different doses of T3 and T4 are injected, 40 μg/kg of T3 and 100 μg/kg of T4 to adult roosters had similar effects on the increase in MHP. These results suggest that T3 is more potent than T4 in stimulating MHP (Astier and Newcomer, 1978; Decuypere *et al.*, 1982a; Mitchell and MacLeod, 1983; MacLeod and Mitchell, 1986, 1989). In birds, T3 possessed a greater TSH suppressing potency than T4 (Gilliland and Strudwick, 1953). In the mammal, T3 is at least six times more potent than an equimolar amount of T4 in preventing goiter, whilst in thiouracil-treated birds T4 was a more potent inhibitor of goitre-formation (Newcomer, 1957). This, however, disagreed with contrasted data reported by Shellabarger (1955) who found that the hormones were equal potent in preventing goitre. The potencies are also equal in stimulating body weight, comb growth, and liver glycogen of propylthiouracil-fed and radiothyroidectomized chicks (Raheja and Snedecor, 1970); in influencing oxygen consumption, heart rate, oxygen deprivation time, and feather growth in thiouracil-treated chicks (Newcomer, 1957); or in suppressing the effect of rapazole, as indicated by thyroid secretion rate (Singh *et al.*, 1968). The turnover rate of the two

iodohormones is similar and so supported the view that T3 and T4 were equally potent (Heninger and Newcomer, 1964). However, one needs to take into account the fact that after T4 injection there was a doubling of the T3 concentration at 10 min postinjection, with T3 returning to the control level at 2 hr postinjection. These changes in T3 and T4 concentrations after T4 injection, are due to peripheral interconversion of these hormones, largely due to 5'-monodeiodination (Astier and Newcomer, 1978; Decuypere *et al.*, 1982b). The T3 injection increased the plasma T3 concentration at 10 min postinjection about 40-fold, and about 7-fold at 2 hr; the T4 injection increased plasma T4 concentration at 10 min postinjection about 90-fold, and even at 24 hr postinjection the T4 values were more than 7-fold above control values (Kittok *et al.*, 1982).

1.2.4. Physiological function of deiodination

There is evidence that the liver 5' monodeiodinase activity is essential for normal growth and development. Injections of GH stimulated embryonic conversion of T4 to T3 (Kühn *et al.*, 1987; Berghman *et al.*, 1989). Furthermore, GH may also inhibit the inactivating pathway of type III 5-monodeiodination activity and therefore may inhibit degradation of T3 to T2 and conversion of T4 to rT3 in chicken's liver (Kühn *et al.*, 1992; Darras *et al.*, 1992a,b, 1993). This inactivating pathway may, therefore, also be important for determining the biological effect of thyroid hormones. These effects would presumably be mediated by binding of GH to its receptor, followed by signal transduction and, ultimately, stimulation of 5'-monodeiodinase activity. This would suggest that GH receptors and signal transduction mechanisms are developed in at least the latter stages of embryonic development.

Several investigations have suggested rT3 blocks the metabolism of T4 to T3 (Chopra, 1978) and numerous studies have found an inverse relationship between T3 and rT3 levels. A change in the T3 : rT3 ratio occurs in chronic malnutrition (Chopra *et al.*, 1975b), acute short term fast (Vagenahis, 1975), anorexia nervosa (Nicod *et*

al., 1976), variety of acute and chronic systemic illnesses including renal and hepatic insufficiency (Chopra *et al.*, 1975b; Chopra, 1976; Nicod, 1976), period following the administration of glucocorticoids (Chopra *et al.*, 1975c), administration of excess T4 in mammals (Chopra *et al.*, 1978) and in birds (May, 1980), and new born period in mammals (Chopra *et al.*, 1975a; Chopra, 1978) and chicks (Thommes and Hylka, 1977).

A new hypothesis by which GH induces changes in peripheral thyroid hormone metabolism has been suggested (Kühn *et al.*, 1993). The liver of the chick embryo contains not only 6-propyl-2-thiouracil (PTU)-sensitive type I deiodinase, but also, in contrast to the mammalian liver, considerable amounts of PTU-insensitive type III deiodinase, with only 5D activity (Darras *et al.*, 1992a). A single GH injection increases plasma T3 and decreases plasma rT3 concentrations in all embryonic stages tested and in chicks immediately after hatch (Darras *et al.*, 1990). Growth hormone may not only stimulate the T3 production *in vitro* by T4 deiodination (5'D-I activity), but also inhibit T3 (and T4) degrading activity (5D-III activity), resulting *in vivo* in increased concentrations of plasma T3 and decreased concentrations of plasma rT3 (Darras *et al.*, 1990; Darras *et al.*, 1992b). Kühn *et al.* (1993) suggested that the regulation of hepatic type III, but not of type I deiodinase, is GH dependent. The question arises which factor might be involved in the control of 5'D-I activity and hence the peripheral production of T3. There is a lack of evidence to support this hypothesis and no reasons for GH regulating 5D-III rather than 5'D-I because T3 is the biologically active form of the thyroid hormone and none of these three enzymes has been purified. In fact, contrary to the situation in chicken embryos, reverse T3 is virtually absent in young posthatch birds, but increases in one to two year old chickens (Premachandra *et al.*, 1977). Hypophysectomy of growing birds decreased 5'-deiodination, an effect which is reversed by treatment with GH. These observations confirm that GH regulates hepatic 5'-deiodination (Darras *et al.*, 1992a; Darras *et al.*, 1992b; Darras *et al.*, 1993; Kühn *et al.*, 1993).

1.3. Hypothalamic and peripheral control of thyroid function in birds

Numerous neuropeptides may act as chemical messengers in the hypothalamo-pituitary axis, but only three: growth hormone releasing factor (GRF), thyrotrophin-releasing hormone (TRH) and somatostatin (somatotrophin release inhibiting hormone, SRIF), are probably of physiological importance in control of thyroid function in birds.

Growth hormone releasing factors have yet to be detected in birds (Harvey *et al.*, 1991a; Harvey, 1993b). Peptides with GH-releasing activity are present in the avian hypothalamus and presumably interact with the avian pituitary receptors stimulated by synthetic (mammalian) GRF preparations (Perez *et al.*, 1990).

SRIF has been shown to inhibit basal GH release and GH release induced by the secretagogues GRF, TRH (Harvey *et al.*, 1991). These actions appear to be mediated by a blockade of cAMP accumulation and impaired protein kinase A (PKA) activity (Donoghue and Scanes, 1991a).

The hypothalamic tripeptide thyrotrophin-releasing hormone (TRH) has been recognised for more than two decades as the major regulator of the biosynthesis and release of pituitary thyrotrophin (TSH) in vertebrates, with the possible exception of teleosts. This is consistent with the fact that thyroxine (T4) is released from the thyroid gland following a TRH challenge. Most circulating triiodothyronine (T3) is derived from T4 by peripheral 5'-monodeiodination (5'D) (Kühn, 1990a). In sheep, and possibly other mammals, TSH also stimulates the intrathyroidal conversion of T4 into T3 and therefore may release T3, prior to T4 (Peeters *et al.*, 1992).

Apart from the release of TSH, TRH stimulates the release of other hypophyseal hormones. Under physiological conditions TRH releases prolactin in

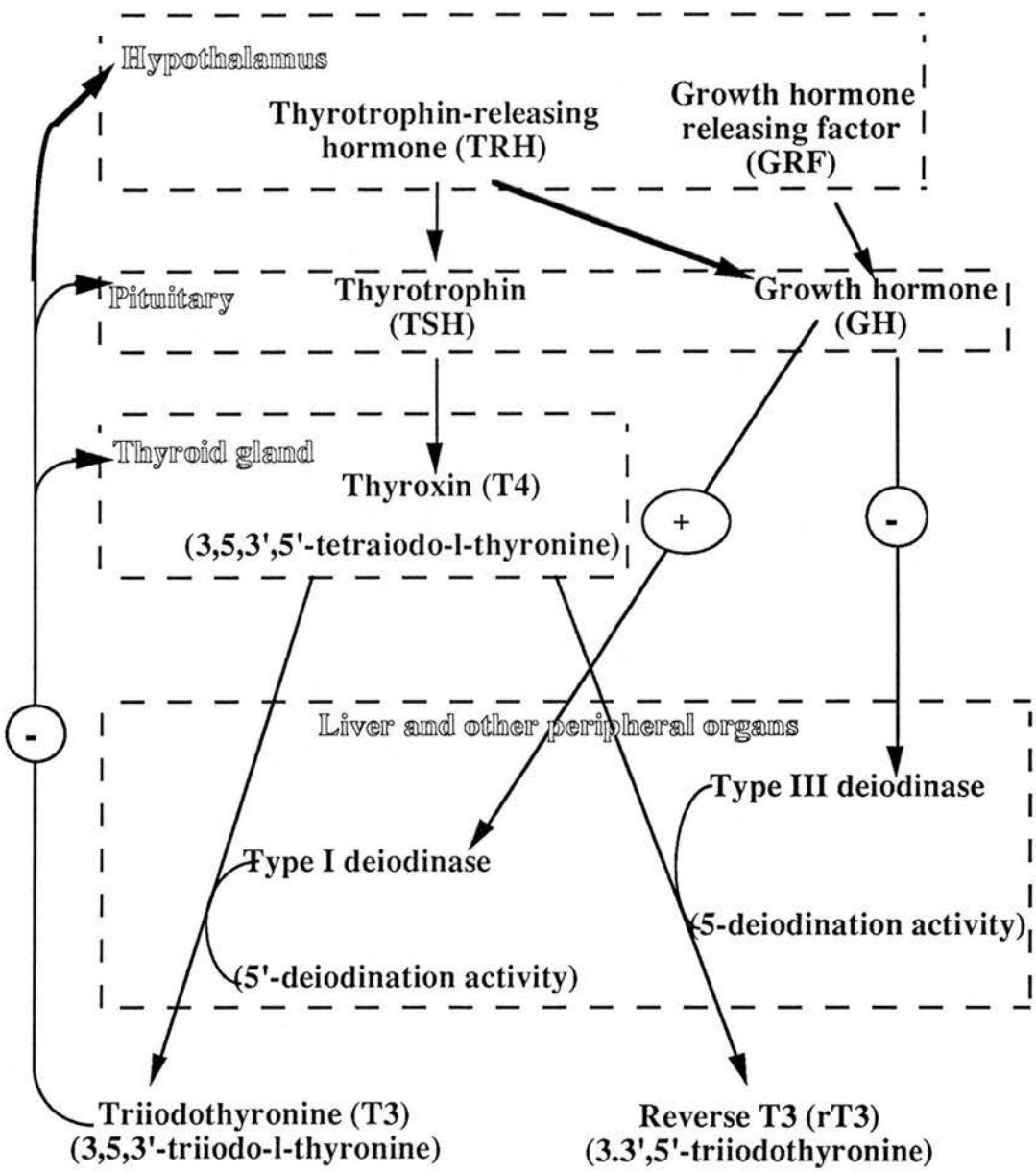
most mammals (Fink *et al.*, 1983), whereas in birds the somatotrophs are stimulated by TRH (Harvey *et al.*, 1978; Harvey *et al.*, 1988). In amphibians, TRH releases prolactin, growth hormone (GH) and α- melanophorotrophin (α-MSH) from the hypophysis together with TSH (Kühn *et al.*, 1990b). In teleosts, no convincing data exist to show that TRH releases TSH, but some results indicate that TRH may be somatotrophic (Wigham and Batten, 1984).

In the chicken, peripheral T3 concentrations are controlled by the simultaneous release of GH and TSH (Kühn *et al.*, 1988a; Berghman *et al.*, 1989), whereas TSH acting alone is purely thyrotrophic and has no direct effect on peripheral T4/T3 conversion (Kühn *et al.*, 1991). On the other hand peripheral fluctuations in thyroid hormones may exert a negative feedback control on both TSH and possibly GH secretion (Harvey, 1990c).

A summary of the mechanisms involved in the control of thyroid function by hypothalamus, pituitary, thyroid, liver and other peripheral organs in birds is shown in Figure 3.

The GH-releasing activity of TRH declines during ontogeny. This age-related diminution of GH responsiveness is probably due to a decline in GH secretory granule volume, somatotroph responsiveness and the number of somatotrophs and TRH-binding sites (Harvey *et al.*, 1991b). A suppression of TRH-induced cAMP, increased metabolic clearance of plasma GH and TRH, and increased sensitivity to thyroidal and SRIF inhibition are also implicated in these ontogenic changes. In old birds, GH is still released in response to exogenous TRH, particularly in birds in which endogenous triiodothyronine (T3) or SRIF are suppressed and GH secretion enhanced by starvation or passive immunoneutralization, respectively (Harvey *et al.*, 1991b; Kühn *et al.*, 1991). Studies on hypophysectomised chick embryos demonstrated that the pituitary gland was necessary for the normal increase in thyroidal activity that occurred between days 10 and 12 of incubation. During this period the sensitivity of

Figure 3. The hypothalamus-pituitary-thyroid axis and the hypothalamus-pituitary-somatotroph-liver axis



T3 is derived almost exclusively from the peripheral conversion of T4. This reaction is stimulated by GH. GH regulates the peripheral conversion of T4 to T3 by the means of stimulation of the liver type I deiodinase (5'-monodeiodination) activity or inhibition of the liver type III deiodinase (5-monodeiodination) activity. The normal functions of the hypothalamus-pituitary-thyroid axis may be regulated by the negative feedback of T3.

the chick embryo to exogenous TRH increased markedly as measured by TRH-induced T4 release. The maturation of a feedback loop had also been indirectly shown to occur during this time interval. (Thommes, 1987). During this period, a low dose of TRH exerted its activity by a thyrotrophic stimulation of the hypophysis as shown by increased concentrations of plasma T4 and reverse T3 (rT3), whereas the release of GH remained unaffected (Decuypere and Scanes, 1983). However, the peripheral mechanisms for T3 production were already present since an injection of chicken GH (cGH) on day 14 of incubation, increased the concentration of plasma T3 and the hepatic T3 production (Darras *et al.*, 1990). In the 18-day-old chick embryo, intravenous (iv) injections of human growth hormone-releasing hormone (hGRH) increased the concentration of plasma GH. This increase was followed by an increase in plasma T3 concentration after two hours postinjection, without affecting the concentration of plasma T4, indicating stimulation of the thyrotrophs and the peripheral conversion of T4 into T3 (Kühn *et al.*, 1988b).

Contrary to the situation in chicken embryos, in the posthatch chicken, the administration of TRH stimulated the incorporation of radioactive phosphate into the thyroid glands (Breneman *et al.*, 1973), the release of ^{131}I from the thyroid gland (Newcomer and Huang, 1974), and increased concentrations of plasma thyroid hormones in juvenile chickens (Kühn and Nouwen, 1978). Increases in T4 concentrations were observed in 3-, 5- and 7-week-old chickens 30 min after a single TRH injection (10 µg/kg body weight), whilst an increase in plasma T3 was observed in 5- and 7-week-old birds 60 min postinjection, suggesting that the administration of TRH stimulated peripheral 5'D activity (Michels *et al.*, 1982). In all birds the T3/T4 ratio in the serum was 10-to 30-times as high as in the thyroid glands during the first two weeks after hatching, suggesting that peripheral 5'D activity was predominantly responsible for the production of T3.

Following injection of 0.1 or 0.25 I.U. bovine TSH/kg into 5-week-old

chickens, a relatively small increase in T3 was observed as compared to T4. Serum rT3 concentrations were also increased after an injection of 0.25 I.U. bovine TSH/kg but the maximal concentration (75 pg/ml) was low (Williamson and Davison, 1986). Reverse T3 was virtually absent in young posthatch birds, but increased in one to two year old chickens (Premachandra *et al.*, 1977).

As for TSH, GH was also released following TRH injection in growing chickens. In both conscious and anaesthetised immature cockerels the administration of 2.5 and 25 μg TRH/kg significantly increased the concentration of plasma GH (Harvey *et al.*, 1978).

The importance of GH in maintaining plasma concentrations of plasma T3 during growth in the chicken was investigated in several experiments designed to lower endogenous GH concentrations. An injection of anti-GH serum into 4-week-old chicks decreased concentrations of plasma T3, compared to controls (Darras *et al.*, 1993). Hypophysectomy substantially reduced concentrations of plasma GH and T3, whereas the effect on plasma T4 was less pronounced. Following injection of GH, an increase in T3 was observed in chicks hypophysectomised for 2 or 3 days, compared to sham controls (Darras *et al.*, 1993). Concentrations of plasma T4 were maintained 3 and 7 days compared to sham operated controls. Therefore, hepatic 5'D-I seems not to be TSH-dependent. Hypophysectomy dramatically increased the number of hepatic GH receptors in hypophysectomised chicks to the numbers measured in sham operated control birds (Vanderpooten *et al.*, 1991).

Negative feedback control by thyroid hormones appears to be very complex, since the amount of TSH and GH released following a TRH challenge is modulated by concentrations of circulating thyroid hormones, particularly T3. Therefore, any condition which influences T3 production may affect both the synthesis and release of GH and TSH.

T3 is also important in maintaining and down-regulating plasma GH released following TRH injection in growing chickens. In both conscious and anaesthetised immature cockerels the administration of 2.5 and 25 μg TRH/kg significantly increased the concentration of plasma GH (Harvey *et al.*, 1978). In the absence of thyroid hormone, GH synthesis is increased (Denver and Harvey, 1991), pituitary TRH receptors are upregulated (Harvey *et al.*, 1991a) and the GH responses to TRH/GRF are potentiated (Harvey *et al.*, 1991b; Tixier-Boichard *et al.*, 1991; Harvey *et al.*, 1991a), indicating inhibitory thyroid actions at post-receptor sites. Thus, as GH increases the peripheral conversion of T4 to T3 (Kühn *et al.*, 1991), thyroid hormones appear to provide inhibitory long-loop stimuli to regulate the hypothalamo-somatotroph axis. They may also regulate circulating GH concentrations by modulating the rate of GH metabolism.

Not much is known about the negative feedback mechanisms controlling TSH release in birds because none of TSH in birds has been purified. Thyroid hormones were reported in quail to decrease the TSH response to TRH by a down-regulation of TRH receptors (Parès-Herbutè and Astier, 1985), and T3 had been found to down-regulate TRH binding sites on chicken pituitary membranes (Harvey and Baidwan, 1990).

Thyroid hormones inhibited basal and both TRH- and GRH-induced GH release from pituitary tissue *in vitro* and *in vivo* (Harvey, 1993a). Thyroxine was less active in this respect than T3 but might exert some of its effects via T3-independent mechanisms, which implied that both thyroid hormones acted at different sites in the hypothalamo-pituitary axis to control the synthesis and release of GH (Harvey *et al.*, 1991a).

Growth hormone was required for a normal growth rate in the chicken since hypophysectomy in young chickens reduces growth while replacement therapy with mammalian GH partially restores growth rate (King and Scanes, 1986). The absence

of a stimulatory effect on growth by GH administration in rapidly growing broiler chicks or turkeys was believed to be due to the high concentrations of circulating GH found in growing birds already exerting a maximal effect on growth, and/or to the number of GH receptors being limiting (Scanes *et al.*, 1993). Results of high plasma GH concentrations maintaining high concentrations of plasma T3 seem to support this hypothesis (Darras *et al.*, 1993).

1.4. Growth hormone and growth

1.4.1. Circulating GH concentrations and growth rate

One approach to delineating the relationship between GH and growth is evaluation of some measure of GH status (e.g., circulating concentrations) among animals differing in growth rate and body size. When normal and sex-linked dwarf (dw gene) lines are compared, pituitary GH synthesis (Hoshino *et al.*, 1982) and circulating GH concentrations (Bowen *et al.*, 1987; Huybrechts *et al.*, 1987; Hoshino and Yamamoto, 1977; Hoshino *et al.*, 1982; Lilburn *et al.*, 1986) were reported to be higher in dwarfs than in normal birds.

When growth-selected lines are compared with slower growing randombred or layer strains, the slower growing lines generally display higher circulating GH concentrations in chickens (Scanes *et al.*, 1980; Burke and Marks, 1982; Stewart and Washburn, 1983; Goddard *et al.*, 1988), Japanese quail (Bacon *et al.*, 1987), and turkeys (Proudman and Wentworth, 1980; Vasilatos-Younken *et al.*, 1988a) during the early post-hatch period. The paradox of lower circulating GH in association with faster growth or larger body size when comparisons are made between lines is not unique to poultry, but is also observed with many species including those for which a large growth-promoting effect of GH has been demonstrated. Gilts selected for rapid growth exhibit significantly lower plasma somatotrophin than slow growth line animals (Norton *et al.*, 1989). It has been suggested that animals utilising

somatotrophin faster will exhibit lower circulating somatotrophin levels (Siers and Swiger, 1971). In support of this, pigs selected for heavier weights at a given age have a higher rate of metabolic clearance of somatotrophin (Arbona *et al.*, 1988), and young, growing chickens have a higher metabolic clearance of GH per unit of metabolic weight than adults (Lauterio and Scanes, 1988). Thus, measures of plasma GH concentration *per se* are not indicative of effective GH status for between-line comparisons. Also, circulating concentrations do not reflect tissue sensitivity, as normal, slow-growing male layer-type chickens exhibit less GH receptor binding at 20 wk of age than faster growing broiler males of the same age (Leung *et al.*, 1987a), and male and female turkeys of a growth-selected line have greater available hepatic GH binding than their slower growing, randombred counterparts at 24 wk of age (Vasilatos-Younken *et al.*, 1990).

In contrast, within lines, GH is often positively correlated with relative gain (growth rate) (Burke and Marks, 1982), particularly when birds are evaluated temporally, such that pituitary GH synthesis (Hoshino and Yamamoto, 1977) and circulating GH decrease as growth rate declines with post-hatch age for chickens (Vasilatos-Younken and Zarkower, 1987) and turkeys (Proudman and Wentworth, 1980; Vasilatos-Younken *et al.*, 1988a). Thus, plasma GH is highest during the early period of most rapid growth and low during slower, late growth. High plasma concentrations of GH are observed during the period of rapid posthatching growth; low GH concentrations are seen in older and adult chickens (Harvey *et al.*, 1979), turkeys (Harvey *et al.*, 1977; Proudman and Wentworth, 1980), ducks (Harvey *et al.*, 1981; Foltzer *et al.*, 1981) and doves (Scanes and Balthazart, 1981). Hepatic GH binding increases with age, concurrent with decreasing plasma GH and growth rate (Leung *et al.*, 1984b; Leung *et al.*, 1987b; Vasilatos-Younken *et al.*, 1990).

Where sexual dimorphism in growth exists, between-sex comparisons of circulating GH concentrations generally yield positive correlations with growth. Males

exhibit both a faster rate and larger body size and higher circulating GH than females, particularly beyond the very early (0 to 3 wk) post-hatch period (Harvey *et al.*, 1977, 1979; Proudman and Wentworth, 1980; Leung *et al.*, 1987a; Vasilatos-Younken *et al.*, 1988a; Johnson, 1988).

1.4.2. The effects of exogenous GH administration on growth rate in pituitary-intact or hypophysectomised chickens

The response of growing, pituitary-intact chickens to GH is perhaps one of the most enigmatic areas of avian endocrinology. In most studies, GH was administered as a daily subcutaneous (s.c.) or intramuscular (i.m.) injection to chickens at hatching or in the early post-hatch period. Among such studies, positive responses have been obtained in only a few cases and for selective GH responses.

The influence of growth hormone (GH) on growth of poultry remains unclear especially in hot weather. Several lines of evidence suggest that it promotes growth in birds. For example, hypophysectomy reduced growth of the body in general (Rosenberg *et al.*, 1963; King, 1969). Further evidence for an influence of pituitary hormones on growth is provided by the report of Bates *et al.* (1962) showing growth in hypophysectomized pigeons was improved by injection with mammalian GH. Growth of autosomal dwarf chickens, but not sex-linked dwarf or normal chickens, was also stimulated by exogenously administered mammalian GH (Marsh *et al.*, 1984). The latter finding is the most direct evidence of a growth promoting effect of GH in chickens. The injection of mammalian GH into normal growing chickens has generally not stimulated body growth (Eaton *et al.*, 1955; Glick, 1960; Sell and Balloun, 1961; Scanes *et al.*, 1984). In contrast, the growth depression of the sex-linked dwarf bird which has an apparent defect in deiodination of T4, so that T3 levels are reduced, is at least partially reversed by supplemental T3. Autosomally recessive birds are dwarf because of some other defect. No clear correlation between thyroid

hormone concentration and growth rate of normal chickens has been identified (King and May, 1984).

Cogburn *et al.* (1989c) reported that, although exogenous chicken GH treatment failed to increase body weight gain, GH injections did increase body fat content to 117% of that of the control group. GH plus corticosterone injections also enhanced the weight of the fat pad above that of controls or hypophysectomy (King and Scanes, 1986). In combination with dietary T3 supplementation, GH, however, greatly reduced carcass fat content above that of T3 alone (Cogburn *et al.*, 1989a). A negative relationship between circulating GH concentration and net fat deposition is also found during the late post-hatch period. Levels of plasma GH from 6-wk of age to sexual maturity were greater overall in broiler chickens selected for low abdominal fat to live weight ratio than in birds selected for high fat (Williams *et al.*, 1986). In contrast to domestic mammals, it is apparent that exogenous chicken GH can not be used to increase lean body mass or improve productive efficiency in chickens. Replacement therapy of GH to hypophysectomized chick embryos partially restored skeletal muscle growth (Vasilatos-Younken and Scanes, 1991), suggesting that GH can influence growth of the chick embryo. In contrast in turkeys, daily i.m. injections of GH (100μg/kg BW) from 6 to 8 wk of age did not alter body weight gain or carcass composition but partially restored plasma IGF-I levels reduced by hypophysectomy in female poults hypophysectomized at 5 wk of age (Proudman *et al.*, 1989).

To identify the more specific effects of individual hormones is more difficult. A 15% reduction in body weight gain over a 3 to 4 week period relative to normal controls was found after young chickens were treated with antibodies to growth hormone to deplete endogenous GH (Groesbeck and Parlow, 1987). Exogenous daily administration of mammalian GH has also been shown to significantly improve body weight gain and bone growth in hypophysectomized chickens, while mammalian

prolactin increased only their body weight gain (Buonomo and Baile, 1988). In fact, chemical thyroidectomy in quail embryos has been observed to decrease growth (McNabb *et al.*, 1984), and thiourea reduced skeletal growth in chick embryos (Hall, 1973). In contrast, body weight gain is more severely affected than bone growth when normal delivery of thyroid hormones to the tissues is limited by goitrogens or thyroidectomy. The greatest effect of goitrogens on growth of embryos occurs during late embryogenesis at a time when normal levels of T3 and T4 are increasing. Posthatching growth is reduced only if the hypothyroid condition is relatively severe. The reduction in body growth does not result from decreased GH level, but the extent to which thyroid hormones influence the action of GH in the chicken or the secretion and action of other growth-promoting hormones or factors has not been established. Exogenous T3 reduces circulating GH level. No beneficial effect of supplemental thyroid hormone has been shown whereas T3 at 1 ppm is detrimental to growth and feed efficiency (King and May, 1984).

The apparent discrepancy in the relationship between circulating GH concentrations and growth for the dwarf likely results from a defect in tissue sensitivity to GH, rather than reflecting the true relationship of GH to growth in poultry. Hepatic GH binding was lower in sex-linked dwarf compared with control line broilers and was undetectable in dwarf layer-strain chickens (Leung *et al.*, 1987a). A deficiency of hepatic GH receptors in dwarf chickens is further supported by the failure of GH to increase plasma T3 concentrations (Kühn *et al.*, 1989).

In mammals, many of the somatogenic or growth-promoting effects of somatotrophin are mediated via the insulin-like growth factors-I and -II (IGF-I and IGF-II), with circulating IGF-I produced mainly by the liver in response to somatotrophin. Immunologically identifiable IGF-I and IGF-I mRNA are also present in extra-hepatic tissues, including epiphyseal growth plate chondrocytes and muscle. Such locally produced IGF-I contributes to the stimulatory effect of somatotrophin on

a number of anabolic processes, including muscle and longitudinal bone growth (Froesch *et al.*, 1985; Daughaday and Rotwein, 1989; and Holly and Wass, 1989). In contrast, muscle growth is reduced by PTU treatment and the growth reduction is reversed by supplemental T4. Total DNA accumulation is reduced in hypothyroid chickens, but muscle mass relative to DNA content is normal following long-term treatment; this suggests some regulation of muscle mass relative to DNA content. T3 increases the number of muscle fiber nuclei in thyroidectomized (Tx) chickens and the uptake of ^{3}H-thymidine into nuclei within the basal lamina (King and May, 1984).

In vitro studies support a role for IGF-I and IGF-II in the growth process in poultry. Insulin-like growth factor-I stimulates thymidine incorporation into DNA of chick fibroblasts (Haselbache *et al.*, 1980), incorporation of proline by embryonic chick cartilage, an increase in weight of the cartilage (Burch *et al.*, 1986), and indices of muscle differentiation in the whole chick embryo (Girbau *et al.*, 1987). Both IGF-I and IGF-II stimulate hepatic glycogen, RNA, and protein synthesis in chick embryo hepatocytes (Widmer *et al.*, 1985). In contrast, T3 directly stimulates growth and maturation of embryonic chick cartilage. There is also evidence that thyroid hormones enhance the *in vitro* action of somatomedins on chick embryo cartilage growth. There is little information concerning the role of the thyroid on posthatching cartilage and bone growth in the chicken. The cartilage growth plate appears more normal in tibias of T3-treated hypophysectomized chickens than in animals not receiving exogenous hormone (King and May, 1984).

In summary, GH does partially restore body weight, skeletal, and specific organ growth (including organs related to immune function) in hypophysectomized poultry, but in the absence (or with deficiency) of other metabolic hormones, does not support optimal growth in the bird. Given the considerable variation among studies in terms of source of GH (pituitary-derived or recombinant), sex of birds used, duration of GH treatment, period over which responses were monitored, dosage administered,

route, and timing of administration, absolute comparison of results is difficult. Overall, GH does not substantially or consistently improve growth performance parameters in early post-hatch poultry when administered by injection or constant infusion, and in some instances, may reduce carcass quality (by increasing fat content).

1.4.3. The effects of exogenous GH administration with intravenous infusion in either a continuous pattern or a pulsatile pattern on growth rate

One approach to delineating the relationship between GH and growth is evaluation of some measure of GH status. It has been established that GH secretion in poultry is episodic, with high-concentration peaks or pulses occurring at regular intervals of approximately 60 to 90 min, superimposed on a relatively constant baseline (Scanes *et al.*, 1983; Cartwright *et al.*, 1984; Buonomo *et al.*, 1984a,b; Vasilatos-Younken and Leach, 1986; Shaw *et al.*, 1987). The magnitude of GH secretion is influenced primarily by the amplitude of secretory pulses and baseline level (i.e., versus pulse frequency or other pattern components). Within lines, secretory pattern characteristics and, in particular, pulse amplitude tend to reflect growth. For example, amplitude as well as baseline concentrations decrease with increasing age and decreasing growth rate in chickens and turkeys (Scanes *et al.*, 1983; Shaw *et al.*, 1987; Vasilatos-Younken and Zarkower, 1987; Johnson *et al.*, 1987). In turkeys, pulse amplitude is higher in males in conjunction with more rapid growth rate than in slower growing females from 4 to 14 wk (Shaw *et al.*, 1987; Bacon *et al.*, 1989). Moreover, in chickens, the amplitude of pulses and baseline may decline earlier in females than in males following the point of divergence in growth rate between sexes (Johnson, 1988).

There is considerable evidence to suggest that the pattern of plasma somatotrophin influences body growth factors in mammalian species such as the rat

and human (Robinson and Clark, 1987; Jansson *et al.*, 1989). Maintenance of a high-amplitude, low-baseline pattern of GH secretion may be essential for effective biological action of the hormone in growing poultry as well. Considering the multitude of factors that contribute to effective GH action (synthesis and secretion, metabolic clearance, target tissue sensitivity, status of other hormones, and growth factors), and the complexity of their interrelationships, it is not surprising that no single measure of GH is consistently and highly correlated with growth.

When GH was administered to late post-hatch (8 to 11 wk) broiler pullets by means of intravenous infusion in either a continuous pattern or a pulsatile pattern (to mimic the frequency and pulse amplitude of 2-wk-old, rapidly growing chicks) growth rate and feed efficiency were improved with pulsatile GH, whereas growth rate was lower and feed efficiency significantly reduced by continuous administration of GH, relative to controls (Vasilatos-Younken *et al.*, 1988b). Measures of carcass fat content were decreased and liver mass increased with pulsatile but not continuous GH, whereas increased plasma IGF-I and measures of longitudinal bone growth occurred under both patterns. Plasma concentrations of several key metabolic regulatory hormones were also markedly different, relative to vehicle-infused controls and between patterns of administration of GH (Vasilatos-Younken *et al.*, 1988b).

The importance of plasma GH pattern relative to the effect of GH on lipid metabolism in chickens was proposed. A pulsatile infusion of GH to female broiler chickens from 8 to 9 wk of age reduced hepatic lipogenic rates over 80% versus control levels, whereas continuous infusion of the same daily dosage had no effect (Rosebrough *et al.*, 1991). Abdominal fat pad weight was concurrently reduced 32% by pulsatile but not altered by continuous GH infusion (Rosebrough *et al.*, 1991). Subcutaneous infusion (chronic exposure) of 50 μg biosynthetic GH/kg body weight per day from 12 to 15 wk of age increased weight of the abdominal fat pad in broiler cockerels (Scanes *et al.*, 1990a). Thus, pattern of exposure to circulating GH

markedly influenced the magnitude and even nature of the response to exogenous GH administration in poultry.

Acute exposure to GH (i.v. injection or pulsatile i.v. infusion) reduces abdominal fat content, and hepatic lipogenic rates (Vasilatos-Younken *et al.,* 1988b; Rosebrough *et al.,* 1991). Chronic exposure to GH (s.c. or i.m. injection or infusion, or continuous i.v. infusion) either has no effect (McGuinness and Cogburn, 1988; Cogburn *et al.,* 1989a; Cravener *et al.,* 1989) or increases carcass fat (Burke *et al.,* 1987; Cogburn *et al.,* 1989b; Scanes *et al.,* 1990b). These divergent responses in lipid metabolism and fat deposition and profiles of insulin and corticosterone with chronic versus acute GH are consistent with the observation that GH plus corticosterone greatly increased abdominal fat pad weight in hypophysectomized chickens, whereas GH alone had no effect (King and Scanes, 1986). This suggests that corticosterone is important to the fattening responses observed with GH administration.

Chronic administration of GH elevates circulating corticosterone (Rosebrough *et al.,* 1987; Vasilatos-Younken *et al.,* 1988b), and numerically increases insulin:glucagon ratios (Cogburn *et al.,* 1989b). Acute administration of GH does not exert these effects (Vasilatos-Younken *et al.*, 1988b; Rosebrough *et al.*, 1991). Corticosterone administration markedly increases carcass fat deposition (Gross *et al.,* 1980; Davison *et al.*, 1983). and *in vitro* hepatic lipogenesis (Kafri *et al.,* 1988). Moreover, corticosterone reduced plasma T3 and IGF-I concentrations in chickens with intact pituitary glands (Buyse *et al.*, 1987). Insulin is required to maintain high lipogenic rates in cultured hepatocytes from fed chickens (Tarlow *et al.*, 1977). Also, insulin inhibits GH-induced lipolysis in chicken adipose tissue *in vitro* (Campbell and Scanes, 1988).

Another possible component of the issue of chronic versus acute exposure to GH is the relative response of thyroid hormones. Although thyroid hormones were also an important parameters on lipid metabolism, pattern of exposure to circulating

growth hormone on thyroid hormones has, however, not yet to be concluded in birds. Acute GH increases circulating T3 concentrations (Kühn *et al.*, 1985; Vasilatos-Younken *et al.*, 1988b), whereas chronic GH has been found to depress levels (Marsh *et al.*, 1984), have no effect (Vasilatos-Younken *et al.*, 1988b), or to increase T3 but to a much lesser degree than acute exposure (Rosebrough *et al.*, 1991). There is strong evidence indicating that T3 is involved in GH action (Cabello and Wrutniak, 1989). In chickens, chronic GH plus T3 supplementation greatly reduced carcass fat content (beyond T3 alone) (Cogburn *et al.*, 1989a).

A pattern emerges from the above studies in which chronic exposure to GH in poultry enhances corticosterone and insulin status and depresses or only modestly elevates T3. Thus, lipogenesis and net carcass fat deposition may be enhanced or unaffected, depending upon GH dosage, duration of GH administration, and the consequent magnitude of metabolic hormone responses that result, as described above. Alternatively, acute GH does not increase plasma corticosterone or insulin concentrations, but does elevate plasma T3. This is associated with reduced measures of carcass fat and hepatic lipogenesis. Given the additional indication that GH stimulates lipolysis in chicken adipose tissue *in vitro* (Campbell and Scanes, 1985), these data collectively suggest that GH can have a positive effect on carcass quality when administered in an appropriate manner (e.g., pulsatile) so as to enhance circulating T3 but avoid concurrent increases in glucocorticoids and insulin.

In addition to the pattern of plasma GH, a second significant factor is the age of the bird at the time of exogenous GH administration. Transplantation of mixed adenohypophysial cells to pituitary-intact female broilers enhanced growth rate (52%) over a 10 wk period when recipients were 10 wk of age at implantation (late post-hatch), but reduced the growth of 2-wk-old (early post-hatch) birds possibly due to feedback inhibition of the endogenous pituitary gland, which was reduced in mass and GH content (Vasilatos-Younken, 1986). Thus, age at application was proposed to be a

key factor in alteration of growth via manipulation of adenohypophysial factors.

Consistent with the studies of Vasilatos-Younken *et al.* (1988b) and Rosebrough *et al.* (1991) in which significant responses to GH in late post-hatch chickens were obtained, biosynthetic GH administered by continuous, s.c. infusion (50 μg/kg body weight per day) from 12 to 15 wk of age increased the growth rate and weight of the *Pectoralis* muscle and bursa, and daily s.c. injections of 250 μg/kg body weight per day from 8 to 11 wk increased the weight and yield of the *Pectoralis* muscle and dressed carcass yield in male broilers (Scanes *et al.*, 1990b).

In conclusion, the available evidence indicates that less than optimal growth occurs in the absence of GH during the early post-hatch period in chickens. However, exogenous administration of GH alone, by current methodologies, to birds with intact pituitary glands during this early developmental period is ineffective in enhancing growth performance. During the late post-hatch period, exogenous GH can enhance growth performance characteristics; however, depending upon treatment protocol and the resulting pattern of plasma GH, carcass quality may be positively or negatively influenced.

1.5. Thyroid function and growth

Evaluation of the effects of hormones on body growth is difficult because growth is a complex process. Body growth curves are sigmoidal and the most rapid growth occurs early in life; the growth rate later slows to a plateau (Batt, 1980). The growth rates of tissues and organs, and the cellular processes underlying their growth, vary with stage of growth. A given hormone may have a greater effect on some tissues or organs than on others and at different stages of growth. Selection of parameters to measure growth is difficult owing to this complexity. Body weight and skeletal size are important parameters but changes in body composition interfere with interpretation of the results. The most obvious changes during thyroid manipulation are changes in

fat and water content.

1.5.1. Changes of thyroid hormone concentrations and body growth curves in the growing chicken

Body growth curves are sigmoidal and the most rapid growth occurs early in life; the growth rate later slows to a plateau (Batt, 1980). In contrast, concentrations of plasma T3 increase up to 2 weeks of age and decrease slowly thereafter, whereas T4 may decrease during the first 2 weeks and increase thereafter (Kühn *et al.*, 1982). A positive correlation exists between relative growth of the chicks and plasma T3, but there is no relationship with the concentration of plasma T4. However, increasing body weight with age is positively correlated with plasma T4 (Kühn *et al.*, 1982). During this period concentrations of plasma GH are high, while hepatic GH receptor availability is low, probably due to internalisation of the GH-receptor complex. An injection of anti-GH serum or hypophysectomy resulted in a decrease in plasma T3.

1.5.2. Thyroid function and body weight and skeletal size in the growing chicken

1.5.2.1. Effect of thyroid deprivation and replacement

Thyroid deprivation significantly reduces body weight gain and bone growth during the second half of embryogenesis. When embryos were treated with methimazole (MMI) from 10 to 19 days of incubation, body weight and the combined length of metatarsus and longest toe (shank-toe) were approximately 65 and 80%, respectively, of control values at 20 days of incubation (King and Delfiner, 1974). The goitrogenic effect was greatest between 17 and 20 days of incubation and thyroxine reversed the growth-retarding effects of MMI on body weight and bone length. Body weight and shank-toe length of embryos treated with MMI from 10 to 19 days of incubation and given 6.0μg/100 gm body weight /day of T4 from 16 to 19

days were 90 to 95% of control values. It is clear that thyroid hormone is essential for normal embryonic body growth especially during the last 4 days incubation. Circulating T4 and T3 levels increase substantially during the last few days of embryogenesis (Thommes *et al.*, 1977; King *et al.*, 1977), when the goitrogenic effects on growth are most noticeable.

Several investigators have demonstrated that severe hypothyroidism significantly reduces posthatching growth in several strains of chickens. When chickens were radiothyroidectomized (RTx) prior to 12 days of age or injected with propylthiouracil (PTU) beginning at 2 days of age, body weights were 40 to 64% of the control values at 5 weeks of age or later, and no strain effect on responsiveness to long term hypothyroidism was observed (Mellen and Wentworth, 1962; Snedecor and King, 1964; Snedecor and Mellen, 1965; Hendrich and Turner, 1966; Snedecor, 1968; King and King, 1976; King *et al.*, 1981). The shank-toe length of the PTU-treated and RTx cockerels were 72 to 77% of the control values, indicating that bone growth was less affected by the hypothyroidism than body weight gain (Snedecor, 1968; King and King, 1976). Information on shorter term (4 weeks or less) effects of RTx and PTU treatment are more difficult to interpret. For example, body weight and shank-toe length were 84 and 88%, respectively, of control values at 4 weeks of age when PTU injections were begun at 2 days of age (King and King, 1973). Since the difference in body weight is much greater after 8 weeks of PTU treatment, one could conclude that hypothyroidism has a lesser effect on body growth during the first month than in the second month after hatching. There is no information, however, concerning how soon the PTU-treated birds become severely hypothyroid. In other studies King *et al.* (1977) have observed that circulating T3 levels are still 64% of control levels at 7 days of age in chickens injected with PTU beginning on the day of hatching, compared to approximately 25% of control values after 5 weeks of PTU treatment (King *et al.*, 1981). Very low doses of T4 (0.25 to 0.30 μg/100g body weight/day) return body growth to control or very near control values in both RTx and

PTU-treated chickens (Hendrich and Turner, 1966; King and King, 1973; Raheja and Snedecor, 1970). This suggests weight gain is depressed only during severely hypothyroid conditions.

Marks and Nix (1973) reported that dietary thiouracil had a greater effect on body weight of chickens selected for rapid growth rate than on nonselected controls. It is unclear whether this difference is due to reduced thyroid function or less effects of hypothyroidism. The body weights of Cornish Cross broilers injected with PTU were 49 to 64% of control values at 4 weeks of age compared to 84% of identically treated White Leghorns (King and King, 1973), suggesting a greater effect of hypothyroidism or goitrogen action during the early growth phase of a heavier strain. More data are needed to determine the role of the thyroid during the early growth phases of different strains of chickens.

Surgical thyroidectomy at hatching may cause a more abrupt reduction of circulating thyroid hormones associated with a reduction in body growth than either RTx or goitrogen treatment (Blivaiss, 1947; Morris, 1951; Snedecor and Camyre, 1966; King and May, 1984). White Leghorn cockerels thyroidectomized (Tx) at 1-3 days of age had body weights that were 65 and 56%, respectively, of control values at 10 and 17 days of age. The shank-toe lengths of the Tx cockerels were 82 and 78% of the control values at 10 and 17 days of age. The average body weight gain of the Tx birds between 10 and 17 days of age was 44% of the weight gained by sham-operated controls, whereas the increase in shank-toe length during the same period was 57% of the control value (King and May, 1984). These results suggest that thyroid deprivation had a greater effect on body weight gain than on bone growth; this effect was also observed in RTx and goitrogen-treated chickens. The body weights and shank-toe lengths of Tx chickens receiving 1.5 or 3.0 μgT3/100g body weight/day beginning the day of thyroidectomy were not significantly different from controls at 10 or 17 days of age. The levels of T3 and growth hormone (GH) were determined in plasma samples

obtained at 17 days of age at approximately 2 hr after the last injection of T3. The average T3 level for 6 out of 8 Tx chickens that had detectable amounts of hormone was less than one-fourth the control value. GH levels were not significantly reduced by Tx but were greatly reduced at 2 hr after the final replacement doses of T3 (King and May, 1984). Harvey *et al.* (1983) observed a similarly low level of immunoreactive T3 in 10-week-old cockerels that had been thyroidectomized at 4 weeks of age, but basal GH levels were significantly elevated in their Tx birds. GH is also significantly elevated in cockerels fed methimazole (Chiasson *et al.*, 1979). Exogenous T4 and T3 greatly reduce plasma GH levels in the intact cockerel, and an elevation in GH acutely follows the administration of TRH (Harvey, 1983). The data show that the reductions in body weight gain and bone growth in the hypothyroid chicken do not result from decreased GH levels. The situation is quite different in rats and other mammals. In contrast, the secretion and circulating level of GH are reduced in hypothyroid rats and are reversed by the administration of T4 (Daughaday *et al.*, 1968; Coiro *et al.*, 1979; Peake *et al.*, 1973). The evidence suggests that thyroid hormones act synergistically with GH in mammals and that thyroid hormone administration causes only minimal stimulation of growth in the absence of GH (Schwartz, 1983). Fisher *et al.* (1982) suggested that thyroid hormones may potentiate GH stimulation of the production and anabolic effects of somatomedins.

Dwarf strains have proved to be useful models in the study of growth and its control. For instance, the sex-linked dwarf chickens and autosomal dwarf chickens have been employed to study the role of GH, T3 and T4 in growth (Scanes *et al.*, 1983). The sex-linked dwarf gene in chicken (male, Z^{dw}/Z^{dw} or female, Z^{dw}/W) and autosomal dwarf gene (dw/dw), are genetic mutations resulting in approximately 35-40% and 50-55% reduced body weight, respectively (Scanes *et al.*, 1983). Both dwarf genes are recessive. The mechanism of dwarfism in autosomal avian dwarfs is still unknown. The low growth rate is not due to a deficiency in GH, and probably not to T4 or T3 deficiencies (Scanes *et al.*, 1983). The mechanism of the reduced growth

in the sex-liked dwarf is likely to be the result of aberrantly low plasma T3 concentrations which in turn are due to depressed peripheral 5'-monodeiodination, but not due to GH deficiency (Scanes *et al.*, 1983).

The extent to which thyroid hormones influence the action of GH in the chicken or the secretion and action of other growth-promoting hormones or factors has not been established. In birds a physiological role for thyroid hormones in the feedback inhibition of GH secretion has been proposed (Scanes *et al.*, 1990a), contrary to the stimulatory effects of these hormones on GH release in mammalian species. T3 treatment *in vivo* (100 μg/kg for 10 days) has been shown to reduce the immunoreactive GH content of the chicken pituitary and to block the *in vitro* GH response to TRH (Denver and Harvey, 1991). Dietary supplementation with T3 was also found to decrease basal and TRH-induced GH secretion in normal chickens and in T3-deficient sex-linked dwarfs (Tixier-Boichard *et al.*, 1991). The ability of dietary T3 to inhibit the GH responses to TRH or GRF in birds in which basal GH secretion is not suppressed (Harvey *et al.*, 1991a) indicates that basal and stimulated GH release might be differentially regulated or have different sensitivities to thyroidal inhibition. The acute exposure of pituitary glands to T3 *in vitro* has also been found to reduce the intracellular content of stored GH (Donoghue and Scanes, 1991b) and the accumulation of newly synthesised [^{35}S] labelled GH (Denver and Harvey, 1991). The synthesis and release of GH induced by TRH, GRF and by activators of the protein kinase A (PKA) and protein kinase C (PKC) second messenger systems is also directly reduced by T3 *in vitro* (Denver and Harvey, 1991; Donoghue *et al.*, 1990a; Donoghue and Scanes, 1991b; Harvey, 1990d). The chicken pituitary gland would, however, appear to be differentially responsive to thyroxine (T4). Unlike T3, T4 does not downregulate pituitary TRH receptors nor inhibit TRH- or GRF- induced GH secretion *in vivo* or *in vitro* (Harvey *et al.*, 1991a).

The high concentration of GH in hypothyroid birds may be partly due to

reduction in the utilization of GH as metabolic clearance rate of GH is reduced in thyroidectomized fowl and restored to that in the controls after T4 administration (Harvey *et al.*, 1990a). The increased production of T3 in GH treated (Kühn *et al.*, 1991) birds might therefore account for their increased rate of GH clearance. The metabolic clearance rate of GH in mammals is, conversely, prolonged by exogenous GH administration (Mullis *et al.*, 1992). The recent demonstration of GH binding proteins in the peripheral plasma of chickens (Vasilatos-Younken *et al.*, 1990; Davis *et al.*, 1992) may, however, also modulate the rate of GH degradation, since protein-binding prolongs the metabolic clearance of GH in mammalian plasma (Herington *et al.*, 1991). An increase in GH clearance and a feedback suppression of endogenous GH release may therefore partly account for the inability of exogenous GH to promote growth in rapidly growing chickens (Scanes *et al.*, 1990c).

These data show that the reductions in body weight gain and bone growth in the hypothyroid chicken do not result from decreased GH levels and at least part of the growth-promoting effects of thyroid hormones in the chicken may not require GH. The body weight gain and increase in shank-toe length of cockerels hypophysectomized at 3 weeks of age were 73 and 56%, respectively, of the values for nonoperated controls at 24 days after hypophysectomy (King, 1969). Thyroxine replacement (1.5 μg/100g body weight/day) returned body weight gain to near normal and the increase in shank-toe length was 81% of the value observed for controls. The hormone was more effective during the first half of the replacement period, when it returned body growth to normal, than it was during the final 12 days of treatment. The increase in shank-toe length of the T4-treated hypophysectomized cockerels was only 33% greater than the increase observed for untreated hypophysectomized birds, and body weight gain was not significantly affected during the last 12 days of hormone treatment.

1.5.2.2. Influence of exogenous thyroid hormones in chickens

The effects of exogenous thyroid hormones on growth have been investigated by feeding and injection. No consistent benefit has been found from feeding iodinated casein (Irwin *et al.*, 1943; Parker, 1943; Newcomer, 1976). These and most similar studies have not reported the absolute levels of T3 and T4 supplied by the iodinated casein. Small doses of injected T4 improved growth rate but a larger dose depressed growth (Singh *et al.*, 1968). Injected T3 increased feed intake (Injidi and Forbes, 1983). Low doses of T3 and T4 in the diet did not give improved growth (May, 1980). T3 at 1 ppm was detrimental to growth rate and feed efficiency, whereas 1 ppm T4 had no effect on body weight or serum T3 concentration. Since T3 decreases plasma GH in young chickens, it is possible that high dose T3 inhibits growth by reducing circulating levels of GH (Scanes *et al.*, 1984).

1.5.2.3. Influence of genetic selection

Extraordinary improvements in the growth rate of meat-type poultry have occurred through genetic selection and several studies have attempted to correlate growth rate with thyroid hormone concentration. Results to date are contradictory and growth rate cannot be predicted from blood levels of T3 and T4. Broilers selected for rapid growth have had both lower T3 concentration (May and Marks, 1983) and higher T3 concentration (Stewart and Washburn, 1983). T4 concentration was not different for strains growing at different rates (May and Marks, 1983). Increased selection pressure for growth increases the difference in male and female body weight but thyroid hormone concentration is not related to sex (Kühn *et al.*, 1982). In contrast, growth hormone levels of small-bodied strains and slow growing randombred strains of broiler chicken were markedly higher than those of large, fast growing strains of broilers (Burke and Marks, 1982).

Sex-linked dwarf broilers have slightly increased T4 concentration and significantly reduced T3 concentration (Scanes *et al.*, 1983; May and Marks, 1983). These changes are probably due to reduced peripheral conversion of T4 to T3 since liver 5'-deiodinase activity is reduced in the sex-linked dwarf (Scanes *et al.*, 1983). The body weights of sex-linked dwarf chickens were increased when they were fed low levels of T3 (Marsh *et al.*, 1983). The autosomal dwarf does not have the reduced deiodinase activity and T3 concentration is not significantly reduced (Scanes *et al.*, 1983). Kühn *et al.* (1982) found a positive correlation between relative growth rate and T3 concentration but not with T4.

1.5.3. Muscle growth

Skeletal muscle growth involves protein accretion and myogenic cell proliferation, and the hormones that affect growth may influence both of these basic biological processes (Allen *et al.*, 1979). It is generally accepted that the number of fibers within a muscle does not increase substantially after birth or hatching and that nuclear numbers increase within myofibers through replication of satellite cells which subsequently fuse with the existing multinucleated fibers (Allen *et al.*, 1979). The degree of fiber hypertrophy during muscle growth should be limited by the increase in myonuclei DNA accretion which precedes or parallels muscle protein accumulation, and there is a close relationship between fiber diameter and the number of nuclei within fibers (Allen *et al.*, 1979; Burleigh, 1977; Knizetova *et al.*, 1972; Moss, 1968). Allen *et al.* (1979) concluded that the accretion of DNA in muscle may be a key factor regulating muscle growth, even though a cause and effect relationship between DNA accumulation and muscle growth has not been proven.

There is considerable evidence that thyroid hormones influence the growth of skeletal muscles in chickens. Gastrocnemius and sartorius muscle growth were significantly reduced in White Leghorn cockerels injected with PTU during the first 8 weeks after hatching, and growth returned to near normal following T4 replacement

(King and King, 1973, 1976). Gastrocnemius fresh weight, total actin content, and total DNA content for the PTU-treated cockerels were 82, 85 and 66%, respectively, of the control values at 4 weeks of age, and 48-53% of the control values at 8 weeks. Since total DNA did not increase in the gastrocnemius muscles of PTU-treated cockerels between 4 and 8 weeks of age, the greater difference in muscle DNA content between control and hypothyroid animals at 8 weeks was due to continued DNA accumulation in the controls. The fresh weight:DNA and actin:DNA ratios for the gastrocnemius muscles of the hypothyroid cockerels were significantly greater than control values at 4 weeks of age but normal by 8 weeks, which suggests some regulation of muscle mass relative to DNA content. Sartorius muscle growth was also reduced in the PTU-treated cockerels, but to a lesser degree than for the gastrocnemius. Total DNA content was 78% of the control value at 4 weeks of age and 60% of the control value at 8 weeks. Sartorius fresh weight and actin content were not affected by the PTU treatment at 4 weeks of age, whereas these parameters were reduced in the muscles at 8 weeks of age to the same degree as DNA content. Pectoralis muscle growth in chickens receiving the same PTU treatment beginning at 2 days of age was affected more than gastrocnemius muscle growth at 4 or 5 weeks of age (King and May, 1984). Fresh weight, fat-free dry weight, and DNA content of pectoralis muscles from PTU-treated Cornish Cross cockerels were 66, 62, 68 %, respectively, of control values at 4 weeks of age. A similar reduction in pectoralis growth was observed for hypothyroid New Hampshire chickens 5 weeks old (King *et al.*, 1981). Although the fat-free dry weight was much higher relative to DNA content in the larger pectoralis muscles of the broiler strain, the muscle mass relative to DNA content in the hypothyroid chickens was normal for both strains after 4 or 5 weeks of treatment. Muscle fresh weight, protein content, and DNA content are also greatly reduced in severely hypothyroid rats (Brasel and Winick, 1970; Cheek *et al.*, 1965). The protein:DNA ratios in the muscles from the hypothyroid rats were approximately 50 and 70 % of control values at 25 days and 6 to 8 weeks of age, compared to the

unchanged ratios observed for the hypothyroid chickens (King and May, 1984).

Thyroid hormones stimulate protein synthesis and degradation in thyroidectomized and hypophysectomized rats (Brown *et al.*, 1981; Flaim *et al.*, 1978; Goldberg *et al.*, 1980). Flaim *et al.* (1978) concluded that thyroid hormones control skeletal muscle growth primarily by acting on protein synthesis, but others suggest that muscle proteolysis is increased in hyperthyroidism and decreased in hypothyroidism and that increased protein degradation may play an important role in decreasing muscle mass in hyperthyroidism (Carter *et al.*, 1981; Goldberg *et al.*, 1980). T3 stimulated muscle protein synthesis in the hypophysectomized rat by increasing both RNA concentration and activity (Brown *et al.*, 1983), but the results of other experiments indicate that a minimal insulin concentration may be required for any stimulation of RNA activity by T3 (Brown *et al.*, 1983).

Studies in chickens, as well as in mammals, provide evidence that thyroid hormones influence total DNA accumulation in skeletal muscle, but there is inadequate information regarding the effect on specific tissue components. T3 stimulates nuclear accumulation and proliferation within skeletal muscle fibers and that T3 response is greater near the ends of fibers than in the middle (King and May, 1984). Exogenous T4 stimulates satellite cell proliferation and the accumulation of myonuclei in muscles of growing rats (Beerman *et al.*, 1983). T4 and T3 also stimulate cellular proliferation in tissues and organs other than muscle (DeFesi and Surks, 1981; DeFesi *et al.*, 1984; Short and Ove, 1983; Truitte *et al.*, 1983).

Although thyroidal influence on muscle growth in the chicken has been most studied after hatching, some information is available for the chick embryo. Gastrocnemius and sartorius muscle weights of embryos treated with methimazole (MMI) during the second half of incubation were approximately half of the control values at 20 days of incubation, and actin levels were only one fourth to one third of the control values (King and Delfiner, 1974). As noted for body weight gain and bone

growth, most of the effect of hypothyroidism becomes apparent between 17 and 20 days of incubation concomitant with large increases in circulating levels of T3. During this 3-day period the muscle weights of normal embryos doubled and total actin levels increased approximately eightfold. There is also evidence that the thyroid influences muscle rRNA metabolism during this growth period (King and King, 1978).

1.5.4. Cartilage and bone

Skeletal growth involves the formation of cartilage that is subsequently replaced by bone. This is particularly evident during growth in length of long bones in which linear growth continues as long as cartilage formation in the epiphyseal growth plate exceeds its replacement by bone. In mammals, thyroid hormones have been shown to influence cartilage formation and the ossification and histology of long bones during their growth and maturation (Schwartz, 1983). However, there is little information available concerning the role of the thyroid on skeletal growth of poultry, other than its influence on bone length noted earlier.

Burch and Lebovitz (1982) demonstrated that T3 directly stimulates growth and maturation of embryonic chick cartilage. Physiological concentrations of T3 increased the weight, length, and total soluble protein content of pelvic cartilages from 9-day-old chick embryos incubated in a serum-free organ culture system. The T3 also stimulated chondrocyte hypertrophy and increased the incorporation of ^{14}C-leucine into protein and $^{35}SO_4$ into the proteoglycan matrix. In an earlier study, tibia weight and length were below normal in hypothyroid chick embryos and contained cartilage in which there was lesser maturation of chondroblasts into chondrocytes, lesser chondrocyte hypertrophy, and insufficient deposition of acid mucopolysaccharide in its matrix (Hall, 1973).

1.6. Thyroid hormone regulation of metabolic heat production and thermoregulation

1.6.1. Thermoregulation and metabolic heat production

1.6.1.1. Thermoregulation

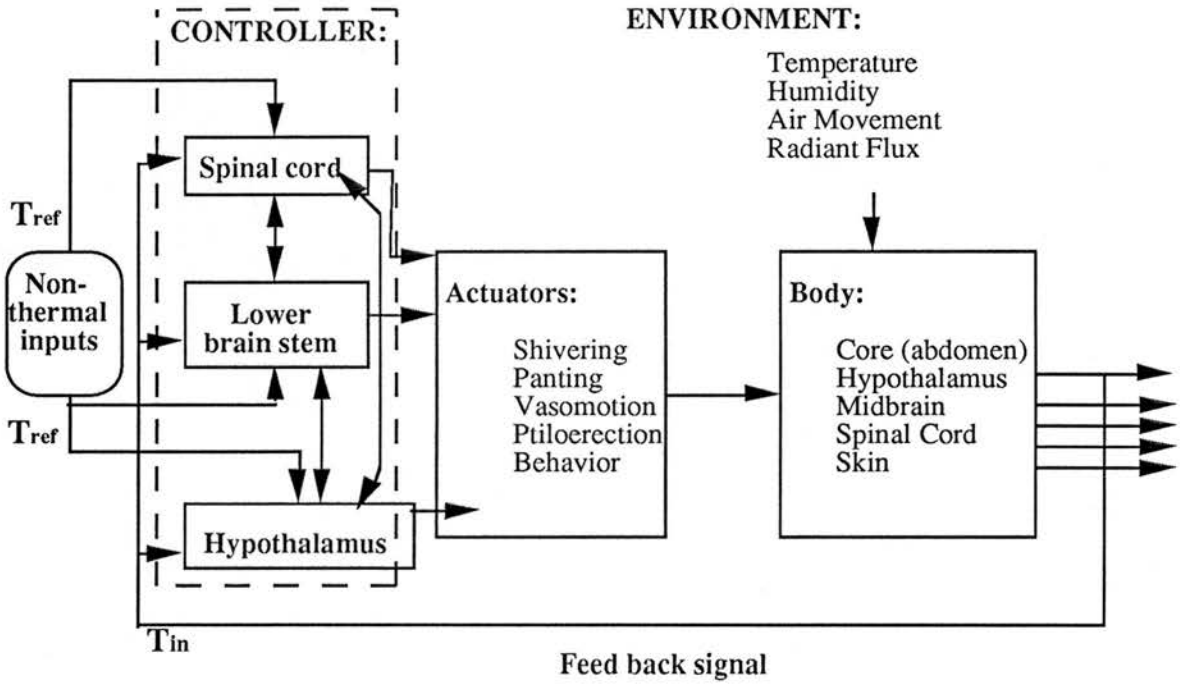
1.6.1.1.1. Thermoregulatory feedback system

Poultry are "homeothermic" in that they maintain a relatively constant body temperature in the face of environmental extremes. In most circumstances both behavioural and physiological responses are involved in temperature regulation.

The complexity and efficiency of thermoregulation is impressive. The concepts of negative feedback control systems, so widely employed in engineering, have been invoked to simplify the presentation of physiological thermoregulatory responses (see Hillman *et al.*, 1985 for review). A simplified schematic diagram of the thermoregulatory feedback system to indicate multiple controllers as well as multiple thermal sensors is shown in Figure 4. An understanding of this control system is most easily accomplished by tracing the signal paths around the diagram. The basis of regulation is the temperature difference between a reference signal (T_{ref}) and the actual controlled thermal inputs (T_{in}). Changes in the thermal environment are sensed by thermoreceptors at numerous points throughout the body and fed to the comparator, where the temperature difference (T_{ref} - T_{in}) yields an "error" signal which drives the thermoregulatory actuators (effectors). This activation of control elements adjusts metabolic heat production (MHP) by affecting shivering or heat loss (HL) mechanisms by affecting panting, vasomotion, ptiloerection, and behavioural responses or both MHP and HL to reduce the temperature difference (T_{ref} - T_{in}).

Evidence by Simon (1974) indicates that these controllers may function both

Figure 4. Schematic diagram describing the temperature regulation system with multiple sensors controllers and effectors in poultry



The diagram indicates that the principle of complexity of thermoregulatory control, and thermal sensory input is composed of multiple feedback signals originating throughout the body. There is substantial evidence to suggest thermoreceptors exist outside of the hypothalamus. Thermal inputs into the thermoregulatory system originate from the spinal cord, midbrain, abdomen, and the skin in addition to the hypothalamus. These controllers may function both independently and in concert with each other. This hierarchical role is indicated by the double arrows connecting the spinal cord and lower brain stem with the hypothalamus while also showing their parallel, independent action. Adapted from Hillman et al. (1985).

independently and in concert with each other. Because the highest precision in temperature regulation is obtained when the hypothalamus is intact, the hypothalamus is considered to perform the role of overall co-ordination. This hierarchical role is indicated by the double arrows connecting the spinal cord and lower brain stem with the hypothalamus while also showing their parallel independent action. Each of the multiple effectors (actuators) may be driven by each of the controllers. It is assumed that the controller system with its multiple inputs influences behaviour. There is an interaction between the autonomic and operand behavioural responses which indicates a preference for specific responses. Figure 4 provides a fundamental framework for understanding the important thermal interactions between the bird and its environment.

The complexity and numerous theories of thermoregulation cannot be adequately covered in detail in this brief presentation, but Figure 4 represents the general characteristics of the temperature regulation system (Hillman *et al.*, 1985).

Based on the large number of experiments with mammals and birds, there is substantial evidence to suggest a multiplicity of inputs, feedback loops, and effector mechanisms (Simon, 1974). Research has shown that thermoreceptors exist outside of the hypothalamus, which for a time was viewed as the only central thermally sensitive region. Studies have shown that thermal inputs into the thermoregulatory system originate from the spinal cord, midbrain, abdomen, and the skin in addition to the hypothalamus (Hillman *et al.*, 1985). Thus, Figure 4 indicates that thermal sensory input is composed of multiple feedback signals originating throughout the body.

According to Hillman *et al.* (1985), behavioural and physiological responses are involved in temperature regulation; the basis of regulation is the temperature difference between a reference signal and the actual controlled thermal inputs.

Various mechanisms exist for birds to protect themselves against the effects of heat. These are (Bianca, 1968):

(a) behavioural mechanisms such as reducing activity;

(b) reducing body insulation in the short term by raising the wings to expose unfeathered areas;

(c) increasing evaporation by an increase in respiration rate, and

(d) lowering heat production

Heat can be transferred from the surface of the bird to the environment by sensible heat loss, i.e. radiation, convection, conduction and also by evaporative means while sensible heat can be transferred both to and from the body, evaporative heat transfer can only occur away from the body. Air movement can reduce the degree of heat stress in birds exposed to high ambient temperatures. Poultry production in hot arid countries may, therefore, be improved (Mitchell, 1986a,b).

Heat loss by evaporation can occur by two routes, through the skin as cutaneous evaporation and through the respiratory tract as respiratory evaporation. At a fixed temperature, evaporation decreases with increasing air humidity and at a relative humidity of 100% when the air is saturated, evaporation normally ceases, i.e. if the air and surface temperatures are identical. However, evaporation is still possible from a bird in an atmosphere saturated with water vapour if the skin and/or membranes of the respiratory tract are at a higher temperature than the air, i.e. a water vapour density gradient exists.

One of the defence responses to heat exposure, panting, requires a change in deep body temperature, at least in chickens (Richards, 1971).

Experimental results from many mammals and birds justify the assumption that each of the multiple effectors (actuators) may be driven by each of the controllers (Simon, 1974). While the quantitative results have not always shown equal effects in thermoregulatory responses, there is sufficient evidence in the literature to indicate a

qualitative influence on each effector by each controller, Poultry do not sweat, although, this response is exhibited in mammals. There also appears to be no simultaneous activation of opposing effector responses (Simon, 1974).

In Figure 4, it is assumed that the controller system with its multiple inputs influences behaviour. The effects of heating and cooling of the spinal cord, hypothalamus, and lower brain stem do influence thermoregulatory behaviour (Baldwin, 1974). Behavioral thermoregulatory responses of animal movement, postural adjustments, huddling, changes in food and water intake, and operant conditioning have been studied. Baldwin (1974) lists some of the behavioural thermoregulatory responses displayed at low and high ambient air temperatures (T_a) by mammals and birds.

Operant conditioning teaches the animal to control the thermal environment by actuating a mechanism (e.g., pecking at a disk) which produces a thermal change in the environment. For example, in a cold environment an infrared lamp can be turned on, or in a hot environment a cool supply of air can be provided for short, timed periods. If behavioural thermoregulatory response are not prevented, each of the controllers (Figure 4) can influence behaviour. Again, the quantitative effects may differ between controller pathways (Carlisle and Ingram, 1973). In addition, the interaction between the autonomic and operant behavioural responses indicates a preference for specific responses. Poultry, when exposed to a cold environment, utilise their species-specific responses of shivering, crouching to cover unfeathered legs, and tucking the head under a wing rather than operantly working for heat reinforcements (Richards, 1976a; Horowitz *et al.*, 1978). Far too little consideration has been given to the effects of confinement housing on behavioural thermoregulation and serious consideration of housing conditions is encouraged.

Although the schematic diagrams of Figure 4 oversimplify the process of thermoregulation. they provide a fundamental framework for understanding the

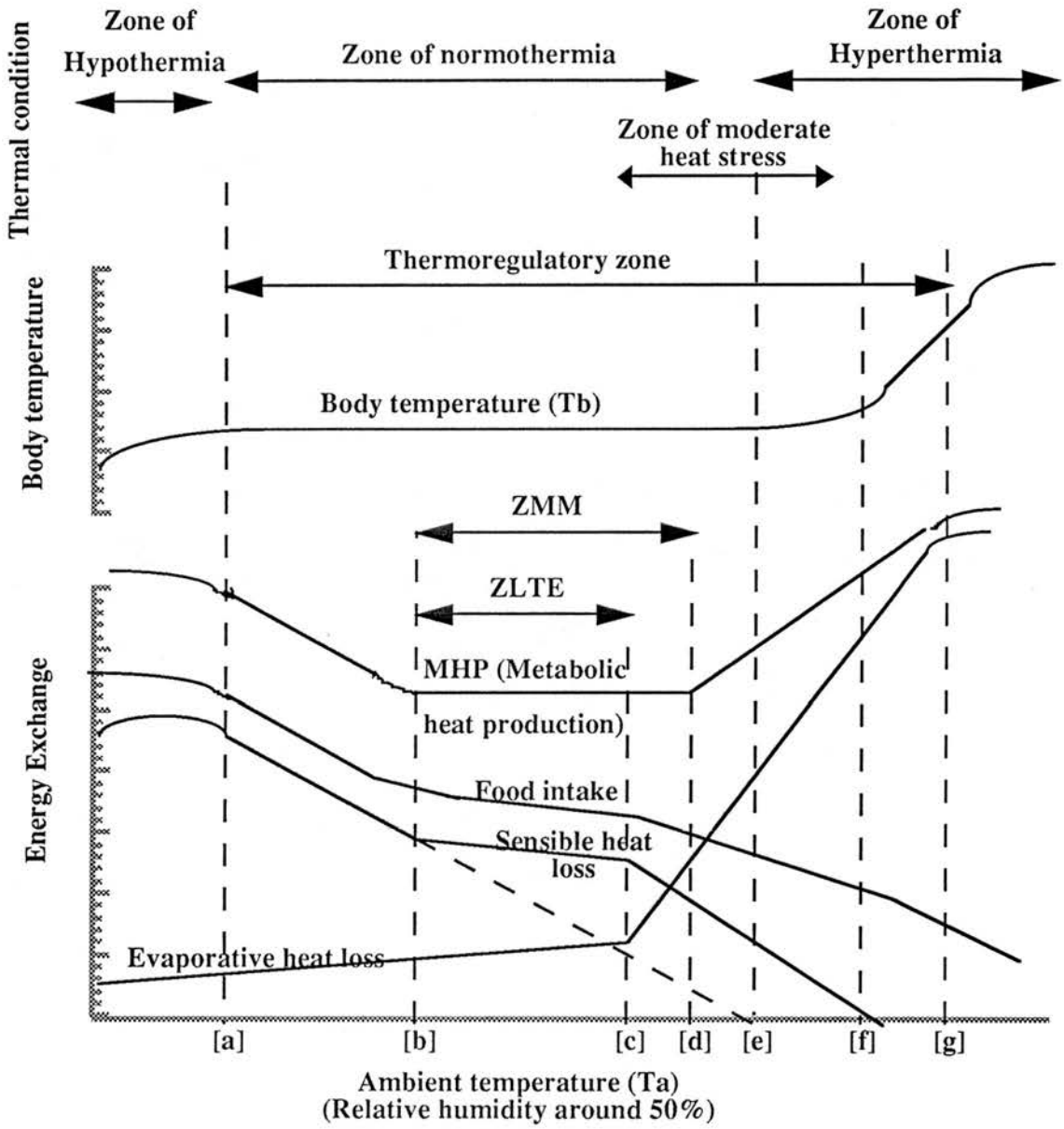
important thermal interactions between the bird and its environment. With these models as a basis one can turn to interactions between the thermal environment and the animal using an energy balance approach. From a practical viewpoint, a poultry-man is said to achieve maximum energetic efficiency of his birds when feed weight is converted into the highest possible body weight of a marketable product. Thus, the direct effect of a cold environment on the energy exchange is to reduce energetic efficiency by increasing heat loss or by increasing food energy intake (Webster, 1974; MacLeod and Mitchell, 1989).

1.6.1.1.2. The body temperature of the fowl

Birds have higher body temperatures than mammals, the deep body temperatures of the common domestic fowl and other domestic species range from 41.2 ° to 42.2 °C in contrast to 36.4 ° to 39 °C for domesticated mammals (Whittow *et al.*, 1964). Smith and Oliver, (1971) reported an average body temperature of the adult fowl to be 41.9 °C. The exact environmental temperature at which body temperature rises above normal depends on air temperature, radiant input, convective exchange, relative humidity of the atmosphere and the degree of acclimatisation, and feeding status of the bird.

Figure 5 shows a generalised schematic diagram illustrating body temperature and partitioning of energy exchange through a wide range of ambient temperature. It illustrates the relationship between metabolic heat production (MHP) and food or energy intake (FI) and to evaluate energy costs required for the process of growth, sensible heat loss (SHL) and evaporative heat loss (EHL) and body temperature, on the other hand, as an increase of air temperature (T_a), in a regime of free convection at 50% RH. It also illustrates the relationships between the energy flows in a generalised sense. Three zones are described. (1) The zone of minimum metabolism (ZMM) is bounded at each end by rising MHP (by point [b], "the lower critical temperature (critical temperature)" where MHP begins to rise as T_a drops and by point [d], the

Figure 5. A model of energy exchange partitioning through a range of ambient temperatures



This generalised schematic diagram illustrates the relationship between metabolic heat production (MHP) and food or energy intake (FI) and to evaluate energy costs required for the process of growth, sensible heat loss (SHL) and evaporative heat loss (EHL) and body temperature, on the other hand, as an increase of air temperature (T_a), in a regime of free convection at 50% RH. It also illustrates the relationships between the energy flows in a generalised sense. Thus, actual values and the relationship between the curves will depend on the species of animal as well as factors of feather quality, acclimation to specific temperatures, season, nutrition status, diet, activity, age, sex, behaviour, and management systems. Adapted from Hillman *et al.* (1985).

"upper critical temperature" where MHP begins to rise as T_a increases). (2) The zone of least thermoregulatory effort (ZLTE) is bounded by the critical temperature (point [b]) at its lower limit and by increasing EHL (point [c]) at its upper limit. The zone of least thermoregulatory effort is usually equivalent to the thermoneutral zone. (3) The thermoregulatory zone is bounded below by the "lower critical temperature" (point [a]), the temperature of incipient hypothermia) and above by the "critical thermal maximum" (point [g], the point at which thermoregulatory function begins to break down). The zone of moderate heat stress in the Thesis is bounded by increasing EHL (point [c]) at its lower limit and above by the point [e] that is between the "upper critical temperature" (point [d], the point at which where MHP begins to rise as T_a increases); and "critical thermal maximum" (point [f], the point at which thermoregulatory function begins to break down). ZMM may be the same as ZLTE in some species and strains in which case points [c] and [d] of Figure 5 are the same. The zone of severe heat stress in the Thesis is bounded by the point [e] and above by increasing T_a until bird died.

Figure 5 also illustrates the relationships between the energy flows in a generalized sense. Thus, actual values and the relationship between the curves will depend on the species of animal as well as factors of feather quality, acclimation to specific temperatures, season, nutrition status, diet, activity, age, sex, behaviour, and management systems (reviewed by Balnave, 1974; Hillman *et al.*, 1985).

According to Romijn and Lokhorst (1966), there is a narrow range of temperature (thermoneutral zone) within which basal heat production by the bird is minimal and body temperature is controlled by variations in heat loss. This range lies between the upper and the lower critical temperatures.

Below the critical temperature, the fowl becomes more active and consumes more feed than when within the thermoneutral zone (Freeman, 1966). Above the upper critical temperature, body temperature increases.

In many countries of the world, particularly in the hot and humid tropical regions, broiler chickens are often maintained at environmental temperatures above the zone of thermoneutrality. In practice, this has a negative influence on performance of the birds due to the physiological and behavioural changes the birds have to institute so as to regulate their body temperature and survive the heat stress.

Sensible heat loss between points [a] and [b] of Figure 5 is assumed to be linear because heat loss is approximately proportional to the temperature difference between the animal and its environment. The total thermal insulation, which is the slope of SHL, is maximal and constant over this range, assuming maximal vasoconstriction and ptiloerection. The actual slope will depend on vasoconstriction, ptiloerection, and behavioural aspects such as postural changes. Within the ZLTE. where panting is not present and MHP is minimal. SHL may not vary linearly with T_a because there is much variation in vasomotor control. Above the upper critical temperature (point [d]) vasodilation is maximal. Thus, the extrapolation of the SHL line between points [a] and [b] intersects with the line of zero energy exchange at point [e] where T_a is equal to normothermic T_b (McLean, 1974).

Evaporative heat loss at low T_a is minimal and determined by the need for respiration and by diffusion of water vapour through the skin. Therefore, MHP must increase, as shown from point [b] to point [a], in order to maintain homeothermy. At high T_a, where MHP is reduced to a minimal level commensurate with maintenance of body processes EHL must increase greatly for HL to equal MHP. The change in EHL between points [c] and [g] must be equal but opposite to that of the SHL in order to maintain energy balance. The intersection of MHP and EHL is at a equal to T_b (point [f]), which is above point [e] because T_b is now elevated above normothermic T_b.

Beyond point [d], the increase in MHP is due to the increased energy costs required for the process of panting and to the effect of elevated tissue temperature resulting from the increase in T_b. In the longer term heat stress a fed animal reduces

feed energy intake which in turn decreases MHP (Farrell and Swain, 1977a, b; Webster, 1974; MacLeod, 1992), but in short-term severe heat stress, MHP might slightly rise comparison with moderate heat exposure due to the increased energy costs required for the process of panting. Oxygen consumption in the Bedouin desert fowl (*Gallus domesticus*) dropped by 8% as T_a increased from 30 to 40 °C, however oxygen consumption increased 18% as T_a increased from 40 to 48 °C (Marder, 1973b). Obviously these birds were severely exposed to high T_a, demanding maximal effort in panting.

Through the range of T_a normally experienced by fowl, direct calorimeter has traditionally been used to measure SHL. Simultaneous measurements of EHL have accompanied SHL measurements so that total heat loss (THL) can be calculated (Ota and McNally, 1961; Jordan and Dale, 1963; Olson *et al.*, 1974; Hillman *et al.*, 1977). In white Leghorns, SHL accounted for nearly 90% of the THL at T_a below about 23 °C (Jordan and Dale, 1963; Walton and Dale, 1963), while in two other studies (Ota and McNally, 1961; Hillman *et al.*, 1977). SHL accounted for about 70 to 80% of THL from fowl within the range of 13 to 29 °C ambient. Preliminary results indicate that the upper limit of the ZLTE (point [c] in Figure 5) may coincide with the upper limit of the ZMM (point [d] in Figure 5) in birds. Misson (1976) suggests that the upper limits of both of these zones (37 °C for Light Sussex chicks) are identical because panting in birds, unlike sweating in mammals, is an active process accompanied by an increase in MHP due to the increased energy costs required for the process of panting. On the other hand, Weathers (1981) shows that EHL does increase well below the upper limit of ZMM for either Japanese quail or Gambel's quail (*Lophortyx gambelii*), thereby following the pattern illustrated by Figure 5. Weathers suggests this may by possible because cutaneous EHL increases by decreasing the vapour pressure gradient through ptilocompression. The energetic cost of small increases in EHL, in addition to fine adjustments in total body resistance within the ZMM or within the ZLTE are needed to more fully understand the

physiological responses of poultry at temperatures and humidities at which they are usually housed.

The thermoneutral condition is the optimal thermal environment which maximises the energetic efficiency based on food energy intake and a marketable product. It is not necessarily true that the optimal thermal environment is synonymous with the optimal environment for thermoregulation. A "system approach" is undoubtedly necessary to consider all of the many questions such as economics, hygiene, labour, disease, etc., together with the thermal environment in order to specify the optimal environment (Hillman *et al.*, 1985).

1.6.1.1.3. Energy Balance

The difference between energy intake and energy loss is the net energy which is utilised by the bird for production, reproduction, and work. The food intake and the gross energy intake will, therefore, increase during cold weather and decrease during hot weather (Van Kampen, 1974). Faecal wastes increase with cold weather and appear to decrease during hot weather. Therefore, the metabolisable energy, which is the gross energy minus the energy of fecal wastes, will change with T_a.

Both direct and indirect methods of calorimeter have been utilised to measure energy metabolism of poultry (Ota and McNally, 1961; Lundy, MacLeod and Jewitt, 1978; Tullett, MacLeod and Jewitt, 1980; MacLeod and Mitchell, 1986). Direct calorimetric techniques measure HL and not MHP, while indirect calorimeter estimates MHP and not HL. However, in most calorimetric studies data are reported when the animal is at thermal equilibrium, therefore MHP equals total heat loss (THL).

1.6.1.2. Metabolic heat production

Birds, like mammals, are homeotherms. In spite of wide environmental temperature fluctuations, deep body or core temperature is maintained within a narrow range by a high but regulated rate of heat production accompanied by controlled heat loss. The maintenance of a homeothermic internal environment therefore depends upon a dynamic equilibrium between heat production and heat loss, i.e.

Heat production = Heat loss + Heat storage

Within the body, heat production is derived from the breakdown of carbohydrates, fats and proteins, i.e. energy input.

The heat store forms a buffer which enables an animal to withstand an imbalance in its thermal equilibrium for short periods. The heat storage capacity per unit surface area is greater in large animals and enables them to tolerate rapid decreases in environmental temperature better than small animals. The maintenance of deep body temperature for long periods is dependent on balancing heat production on the one hand with heat loss and heat storage on the other. Thus deep body temperature is a function of heat production, heat loss and heat storage.

Heat is produced by oxidation processes in the active protoplasm of the body. Muscle and glands are the most active tissues of the body and are therefore the principal areas of heat production (Hill, 1988). Heat is produced in the body in several ways. The most important forms of heat production are basal heat production for maintaining essential body processes, like deep body temperature and cardio-respiratory activities; digestive heat production, this varies with the availability and quality of food that is provided; muscular heat production, which is controlled by the physical activity of the animal; increased metabolism due to productive processes, such as growth and egg production (McDonald *et al.*, 1984).

The means by which chickens can vary their heat production are limited. They can reduce productive processes and muscular activity and to a more limited extent digestive heat production by a reduction in food intake, but they can not normally reduce basal heat production as minimal body processes must be maintained (Smith, 1990; Payne, 1990).

1.6.2. Control of thermoregulation by hypothalamus

The centres responsible for heat balance control are located in the hypothalamus. They consist of two centres. The heat-loss centre is situated in the anterior hypothalamus, whilst the heat production centre is in the lateral hypothalamus (Amakiri and Heath, 1985). The mammalian and avian hypothalamus contain relatively large amounts of the monoamides, noradrenaline, adrenaline and 5-hydroxytryptamine. It has been suggested that their presence or relative concentrations may be related to temperature regulation (Freeman, 1971a).

The heat loss centre is activated by the rise in the temperature of the blood bathing its cells. When the blood is cooled, the heat conserving area is stimulated and the body heat is retained, or more heat is produced. The reverse takes place when the blood is warmed. The heat loss centre is also activated by reflexes from the thousands of receptors in the skin.

The centres responsible for growth control are also located in the hypothalamus. They consist of several hormones controlling growth, heat production and syntheses of proteins. The hypothalamus of the domestic fowl contains TRH (Jackson and Reichlin, 1974) while *in vitro* TRH release for the chicken hypothalamus is increased by synthetic TRH and extracts of avian hypothalami (Scanes, 1974). Furthermore, TRH increases avian TSH secretion *in vivo*, as indicated by elevated circulating concentration of T4 (Klandorf *et al.*, 1978; Kühn and Nouwen, 1978; Campbell and Leatherland, 1979). The addition of T3 to the diet of chickens is

followed by a dramatic decrease in the plasma concentrations of T4 (May, 1980). this presumably indicates that T3 has a negative feedback effect on TSH secretion. TRH to be an effective GH secretagogue in poultry (Vasilatos-Younken and Scanes, 1991).

The hypothalamus occupies a key position in the neuroendocrine control of the anterior pituitary, serves a key function in autonomic and behavioural homeostatic mechanisms. Among these homeostatic mechanisms is the control of body temperature.

The hypothalamus is a complicated network which receives information, processes and integrates it, and sends out signals either to parts of the nervous system (central and/or autonomic) or to organ systems, so that the animal can respond to disturbances of its external or internal environment. Substantial amounts of information are available concerning inputs into the hypothalamus and about the biochemical aspects of its function for mammalian species (see Boulant, 1980 and Myers, 1980). In spite of this rather voluminous information, Stitt (1981) has stated: "A major obstacle to studying the neurophysiology of thermoregulation and fever is the absence of an obvious correlation between neuroanatomy and function in the hypothalamus. Present methods of identifying and classifying hypothalamic cells as participants in thermoregulation are patently inadequate."

Considering that the information about the avian hypothalamus is fragmentary in comparison with the information about the mammalian hypothalamus in its thermoregulatory function, this review can only hint at possible mechanisms and relationships.

The evidence presented so far on the function of the hypothalamus as a controller on temperature regulation of birds is more suggestive than convincing. The data do indicate strongly that the hypothalamus is part of a central nervous network. It is, of course, possible that evidence obtained through other methodologies will shed

more light on the control function of the avian hypothalamus in regulation of T_b.

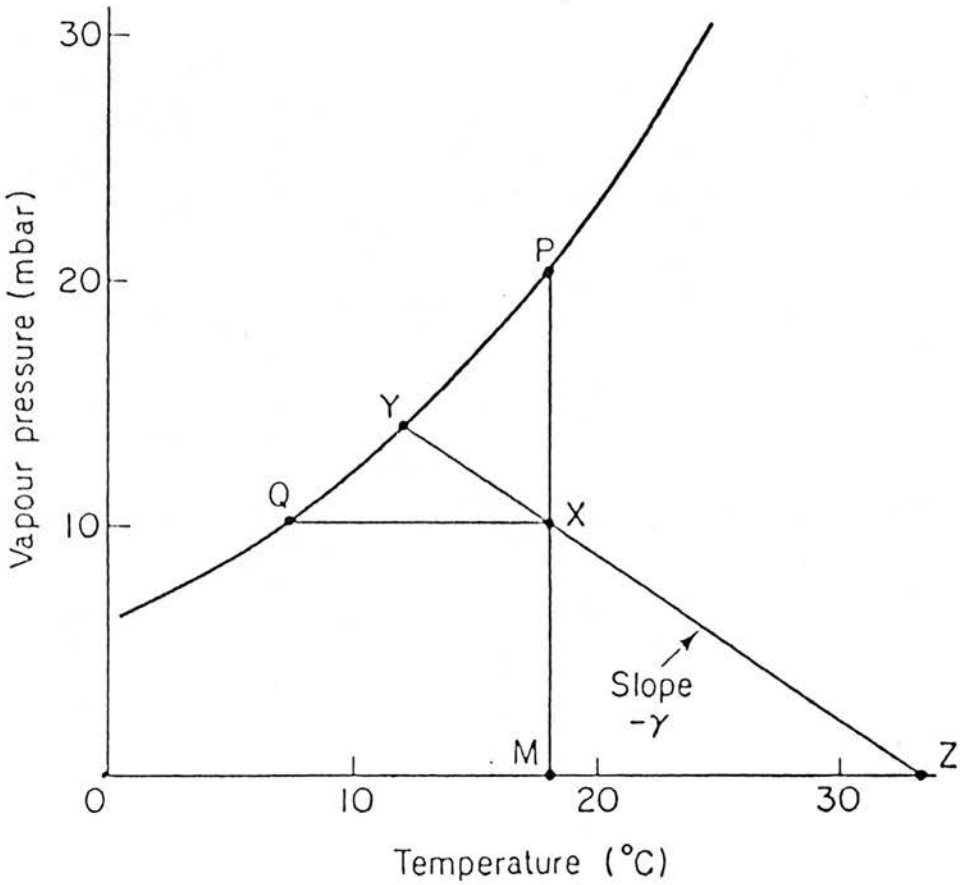
1.6.3. Definition of thermal environment

As environmental temperature rises the temperature gradient between the bird and its environment decreases with a subsequent decrease in sensible heat loss, the bird comes to rely increasingly on the evaporative heat-dissipating mechanisms in order to maintain its body temperature within the normal range. This is because the temperature difference between the body surface and the outside is small, under these circumstances the evaporative heat loss is the only avenue which does not depend on temperature differences (Hsia, 1990). Heat loss by evaporation can occur by two routes, through the skin as cutaneous evaporation and through the respiratory tract as respiratory evaporation. As humidity increases at high environmental temperature, heat loss from the chicken's respiratory tract in the latent form becomes progressively more important, the birds have to be panting more severe to loss the same amount heat as those at lower relative humidity. At a fixed temperature, evaporation decreases with increasing air humidity and at a relative humidity of 100% when the air is saturated, evaporation normally ceases, i.e. if the air and surface temperatures are identical.

Heat stress is usually described in relation to elevated dry bulb temperatures but water vapour density (VD) also has important effects upon heat exchange under such conditions. The environmental condition should, therefore, be well defined by heat load rather than temperature alone. Figure 6 gives the complex relations between dry bulb temperature, wet bulb temperature, equivalent temperature, vapour pressure (water vapour density) and dew point.

The environmental conditions could be defined by application of the concept of an apparent equivalent temperature to measure heat loads as suggested by Monteith and Mount (1973). The apparent equivalent temperature is a defined by the following equation:

Figure 6. The relation between dry bulb temperature, wet bulb temperature, equivalent temperature, vapour pressure and dew point.



The point X represents air at 18 °C and 10 mbar vapour pressure. The line YXZ with a slope of -γ gives the wet bulb temperature from Y (12 °C) and the equivalent temperature from Z (33.33 °C). The line QX gives the dew point temperature from Q (7.1 °C). The line XP gives the saturation vapour pressure from P (20.6 mbar). The ratio of lines XM:PXM in percentage represents relative humidity (RH). If a psychrometer constant γ is replaced by : $\gamma^*=\gamma(r_v/r_H)$, then:
an apparent equivalent temperature (θ^*) = $T+e/\gamma^*$
Adapted after Monteith (1973).

$\theta^* = T+e/\gamma^*$

where θ^* is an apparent equivalent temperature; T is absolute temperature; e is partial pressure of water vapour in air; γ^* is an apparent value of psychrometer constant defined by:

$\gamma^*=\gamma(r_v/r_H)$

where r_H is the appropriate diffusion resistance for heat transfer (i.e. sensible heat); r_v is the appropriate diffusion resistances for heat transfer by evaporation. γ is a psychrometer constant defined by:

$\gamma= c_p p/\lambda\varepsilon$

where c_p is the specific heat of air at constant pressure; p is the water vapour density; λ is latent heat of vaporisation of water; ε is ratio of molecular weight of water vapour and air (Monteith, 1973).

1.6.4. Thyroid hormone and thermoregulation

There is evidence in chickens that thyroid hormones regulate metabolic heat production (MHP) and body temperature (Klandorf *et al.*, 1981; Astier and Newcomer, 1978; Decuypere *et al.*, 1982a; Mitchell and MacLeod, 1983; MacLeod and Mitchell, 1986, 1989) and promote oxygen uptake in chick myocardium (Newcomer and Barrett, 1960). In birds and mammals thyroid hormones play a role in heat production and in the metabolism of carbohydrates, proteins and lipids. Early studies failed to show any effect of thyroid hormones on the general metabolism of poikilothermic vertebrates (Etkin and Gona, 1974) but it was subsequently shown that in amphibians and reptiles, the general metabolic response of the tissues to thyroid hormones is dependent on temperature (Maher, 1965, 1967; Packard and Packard, 1975; Hulbert, 1978). Thus thyroid hormones have a calorigenic effect in lizards

when they are at 30 °C but have no measurable effect at 20 °C. These effects are initiated in the nucleus, but are eventually expressed by alterations in mitochondrial and membrane activity and appear to be reversible (Oppenheimer, 1979; Hulbert 1978). Fasting MHP declined in heat-acclimated fowl (Klandorf, Sharp and MacLeod, 1981), which may be due to a drop in thyroid activity (Shafie *et al.*, 1979; MacLeod and Mitchell 1989).

1.6.4.1. Thyroid function and metabolic heat production

In birds hypophysectomy leads to a decrease in the size of the thyroid gland. Sectioning the hypophyseal portal vessels also results in a reduction in the weight of the thyroid gland (Rosenberg *et al.*, 1967) and it was concluded that the activity of avian thyrotrophs are partially dependent on hypothalamic control. Comparable studies in mammals have shown that hypophysectomy results in a decrease in metabolic rate. food intake, heart rate and body temperature. Riddle *et al.* (1935) measured a significant drop in the basal metabolic rate of pigeons after hypophysectomy. Similar findings were also reported for other avian species including the Japanese quail (Woitkewitsch, 1940) and the domestic fowl (Keating *et al.*, 1945).

Thyroid hormone play a major role in regulating oxidative metabolism of birds. T3, the metabolically active thyroid hormone, plays an active role in energy metabolism and metabolic rate (Klandorf *et al.*, 1978, 1981). Any pronounced alteration in thyroid function is reflected in an altered metabolic rate. According to Klandorf *et al.* (1981), there is a higher correlation between plasma T3 concentration and metabolic heat production, than between plasma T4 concentration and metabolic heat production. In birds and mammals thyroid hormones play a role in heat production and in the metabolism of carbohydrates, proteins and lipids. A reduction in the levels of plasma T3 induced by thyroidectomy, fasting or by an increase in environmental temperature was associated with a decrease in the rate of heat production (Klandorf, 1982). An injection of T4 into thyroidectomized birds resulted

in a sustained increase in the levels of plasma T3 (Klandorf, 1982). When exogenous doses of T3 and T4 are given to fasted Leghorn roosters, plasma T3 concentration is higher than control, for 24 hr (Kittok *et al.*, 1982). Heat loss after either T3 or T4 treatment did not differ, but treatment with either hormone resulted in a heat loss higher than that of control. The coefficient of correlation between oxygen consumption and T3 or T4 concentration in the plasma in different age groups of White Rock chickens ranged between 0.78 and 0.98 (Bobek *et al.*, 1977).

1.6.4.2. Thyroid hormones and body temperature

In birds and mammals the thyroid apparently has a principal function of maintaining a normal MHP. Thyroidectomy or administration of inhibitors of thyroid hormone synthesis such as thiouracil or thiourea results in an inadequate temperature regulation at low T_a and a depression of MHP (Nobukuni and Nishiyama, 1975; Freeman, 1971b; Assenmacher, 1973). Thyroid function parallels the time course of developing thermoregulatory ability in Japanese quail (Spiers *et al.*, 1974). In the neonate chicken, T3 or T4 (300 μg/kg) injected intraperitoneally is thermogenic (Freeman, 1970). Rectal temperature was elevated within 30 min but was significantly higher only in those chicks given T3 at 60 min after treatment. Thiouracil impairs thermoregulation in the neonate chicken (Freeman, 1971b). Administration of either T3 or T4 (both are hormones sermons secreted by the thyroid) to baby chicks, increases their ability to maintain body temperature (T_b) upon exposure to 20 °C, a low T_a for chicks (Freeman, 1970). Chicks thyroidectomized at 12 days and exposed to 30 °C had a T_b 0.4 °C lower than controls ($p<0.001$) and also a lower MHP ($p<0.001$); at T_a of 12 °C the T_b was 0.7 °C lower than in controls, but MHP was not significantly different from controls; however, the respiratory quotient (RQ) was lower, 0.93 vs. 0.98 for controls. Administration of T4 did not improve the poor thermoregulation of thyroidectomized chicks kept at 5 °C (Davison *et al.*, 1980). Davison *et al.* (1980) ascribed this lack of an effect of T4 to the poor feathering and

poor nutritional status of the thyroidectomized chicks. It is unfortunate that the effect of T3 was not also investigated.

Feeding of either T3 or T4 to 6- to 7-week-old broilers for 8 to 26 days diminished the survival time when exposed to high T_a (40 to 42 °C) at a relative humidity of about 80% (May, 1982). Adult roosters exposed to high T_a (42.2 °C) survived longer than controls if they had been fed thiouracil and less than the controls if they received thyroxine (Fox, 1980). The secretion of T3 and T4 is under the control of the anterior pituitary by its secretion of thyroid-stimulating hormone (TSH). The avian anterior pituitary-thyroid unit is more autonomous with respect to its control by the hypothalamus than the mammalian anterior pituitary-thyroid unit (Assenmacher, 1973). In view of this relative independence, it would be interesting to determine the effect of intra-ventricular thyroid-stimulating hormone-releasing hormone (TRH) infusion on thermoregulation of birds. In cats, infusion of nanogram quantities of TRH into the third ventricle is followed a pronounced hypothermia, with the extent hypothermia being related to the dose (Metcalf, 1974). This hypothermia is the result of tachypnea induced by TRH and is not the result of an effect on thermoregulatory hypothalamic neurones (Myers, 1980).

Consideration of experiments involving either the effect of temperature on thyroid hormone secretion or the effect of thyroid hormone administration on MHP or temperature regulation must distinguish between acute and chronic experiments.

T3 and T4 have different relative potencies when different function are evaluated. In the goiter prevention test in which thiouracil is administered and different doses of T3 and T4 are injected, 0.0064 μmol/100 g/day of T3, but 0.0052 μg/100 g/day of T4 were required to obtain thyroids of similar weight as those of untreated controls (Mellen and Wentworth, 1959). Thus T4 was more potent than T3 in this test. The relative potency of these hormones with respect to stimulation of MHP needs to be investigated in more detail. In an acute experiment with adult roosters, 40 μg/kg

of T3 and 100 μg/kg of T4 had similar effects on the increase in total SHL and neither hormone affected O_2 consumption and CO_2 production. These results suggest that T3 is more potent than T4 in stimulating total SHL. However, one needs to take into account the fact that after T3 injection the T4 concentration in the plasma was higher for the first 8 hr after injection than it was in control birds, and after T4 injection there was a doubling of the T3 concentration at 10 min postinjection, with T3 returning to the control level at 2 hr postinjection. These changes in T3 and T4 concentrations after T4 and T3 injections, respectively, are due to peripheral interconversion of these hormones, largely due to 5'-monodeiodination (Astier and Newcomer, 1978; Decuypere *et al.*, 1982b). The T3 injection increased the plasma T3 concentration at 10 min postinjection about 40-fold, and about 7-fold at 2 hr; the T4 injection increased plasma T4 concentration at 10 min postinjection about 90-fold, and even at 24 hr postinjection the T4 values were more than 7-fold above control values (Kittok *et al.*, 1982). Neither the T3 nor the T4 injections affected O_2 consumption or CO_2 production in these experiments.

Using baby chicks, Singh *et al.* (1968) in an acute experiment, reported no effect of 4 μg/100 g of T3, or 5 μg/100 g of T4 or of the combination of both on MHP. In a chronic experiment in which 6 μg/100 g of both T3 and T4 were injected for 15 hr where MHP was measured at 2,3, and 24 hr after the last injection, a statistical but not significant transitory increase in MHP occurred. However, at 24 hr there was a significant depression in this parameter after the T3 and the T3 + T4 injections. This decrease is probably the result of an inhibition of TSH secretion by the pituitary.

Arieli and Berman (1979) using adult laying hens, found after 7 days of treatments with four different doses of T4 (0, 10, 30, and 100 μg/kg), that 30 and 100 μg/kg T4 increased O_2 consumption significantly when the measurements were made at 4 °C. Treatments of 30 and 100 μg/kg of T4 increased O_2 consumption significantly

when measurements were made at T_a of 21 and 32 °C. Unfortunately, no data were collected concerning the effects of T3 injections; none of the doses of T4 affected T_b.

Measurements of plasma T3 and T4 concentrations have, with one notable exception, yielded higher concentrations of T4 than of T3 (Sadovsky and Bensadoun, 1971). The ratio of T3/T4 reported in the literature varies between 0.05 and 0.46, depending on the age and sex of birds used, the time of day of the measurement, and the reproductive stage of the birds (Klandorf *et al.*, 1981; May, 1982; Kittok *et al.*, 1982; Newcomer, 1974; May, 1974; May, 1978; Bobek *et al.*, 1977; Kühn and Nouwen, 1978; Klandorf *et al.*, 1978; Klandorf *et al.*, 1982). The value of T3 and T4 concentrations reported by Sadovsky and Bensadoun (1971) for White Leghorn roosters is one order of magnitude higher than those reported by the other investigators cited above, whereas the values reported for T4 concentrations are about twice as high. One reason for such a difference may be a difference in techniques used, although a tenfold difference remains difficult to explain.

Both T3 and T4 concentrations in the plasma of chickens show daily variations, but the hormones are out of phase with each other between 4 and 12 hr, depending on the physiological status of the birds. For instance, in White Leghorn cockerels the peaks of T3 and T4 concentration (acrophase) occur at 10.16 and 0.47 hr after lights on, respectively. In bantam hens, the phase difference is 7.37 hr for laying, 4.22 hr for incubating, and 8.09 hr for hens brooding chicks. These daily rhythms are apparently phased by the feeding pattern of the birds (May, 1978; Klandorf *et al.*, 1981). If the secretion is indeed phased by the feeding pattern, then the question still arises: why are the T3 and T4 peaks out phase with each other if the secretion is controlled by TSH secretion by the pituitary? Experiments by Decuypere *et al.* (1982b) suggest that the ratio of T3 and T4 in the plasma is the result of the interaction between thyroid hormone secretion, principally T4, and 5'-monodeiodination outside the thyroid, the former process being under the control of

TSH and the latter being modulated by food intake.

A daily rhythm has also been observed in domestic ducks under 12L:12D; the acrophase for T4 falling in the dark and the one for T3 falling during the light period (Harvey *et al.*, 1980).

In adult Japanese male and female quail, transfer from T_a = 22°C to T_a = 34 to 35 °C resulted in a 25% increase in T3 at 6 hr and a return to the base level at 10 hr followed by a steady increase to 50 hr when the experiment was terminated, whereas T4 concentrations declined steadily from 18 ng/ml at the of transfer to 10 ng/ml at 50 hr (Bobek *et al.*, 1980). As expected, O_2 consumption increased during cold and decreased during warm exposure in these experiments. It appears from the data that during cold exposure there is a correlation between O_2 consumption and both T3 and T4 concentrations in plasma, but during exposure to a warm environment, T4 concentration and O_2 consumption seem to be positively correlated, whereas T3 concentration and O_2 consumption are negatively correlated.

Baby female chicks exposed to T_a = 7.2 °C from day 8 or to T_a = 20.0 °C from day 2 showed higher plasma T3 concentrations (tested at 35 days of age) than female chicks exposed to T_a = 32.2 °C from day 1 in both of two experiments. The female chicks exposed to T_a = 32.2 °C had two higher T4 concentrations than in the other two groups of female chicks in one of two experiments, whereas in the other experiment differences were not significant. For male chicks, the T3 concentration was lower in the chicks exposed to T_a = 32.2 °C than in those exposed to T_a = 7.2 or T_a =20.0 °C in one experiment, whereas no significant differences were found in the other experiment. For T4 concentrations in one experiment, the values obtained in the chicks exposed to T_a = 20.0 °C were lower than those obtained in the T_a = 7.2 °C and the T_a = 32.2 °C groups, with no significant differences found in the second experiment (May, 1974).

In experiments with fasted and fed laying hens, Klandorf *et al.* (1981) found after exposure to T_a = 32.2 °C that (1) the daily variation in T3 and T4 concentrations was more pronounced at T_a = 32 °C that at T_a 20 °C, (2) T3 concentrations in plasma were consistently higher in birds kept at T_a = 20 °C than in birds kept at T_a = 32 °C. The T4 concentration in the plasma was lower in the fed birds at T_a = 20 °C than in the birds at T_a = 32 °C for four of the five time periods considered; for the fifth period the concentration of T4 in the two groups was similar, (3) heat production of the fed birds was lower at T_a =32 °C than at T_a = 20 °C. These data suggest a higher correlation between plasma T3 concentrations and MHP that between plasma T4 concentration and MHP. Bobek *et al.* (1977) had found in a study of age on MHP and T3 and T4 concentrations in plasma that, T3 concentrations were highly correlated (r = 0.78 to 0.98) with O_2 consumption, whereas T4 concentrations and O_2 consumption showed variable correlations (r = -0.63 to + 0.13).

Based on such studies and investigations in which T3 and T4 were administered, it appears that T3 serves an important function in the regulation of MHP and temperature regulation, especially when long-term adjustments to exposure to low or high T_a are considered. T3 also appears to be a more potent hormone than T4 in stimulating metabolism. Bilezikian *et al.* (1980) observed an increase in body temperature (41.0 °C) in mature turkeys made hyperthyroid, and reduced body temperature (39.8 °C) in those made hypothyroid.

1.7. The conflicting reports of changes in thyroid hormone levels in heat stressed birds

1.7.1. Stress

The word "stress" is very subjective and it can mean different things to different people, probably one of the best definitions reported. Fraser *et al.* (1975) proposes that an animal is said to be in a state of stress if it is required to make

abnormal or extreme adjustments to its physiology or behaviour to cope with adverse aspects of its environment and management. A husbandry system can be said to be stressful if it makes abnormal or extreme demands on the animals.

It is widely acknowledged that in many cases, animal production is reduced by "stress" imposed on the animal by environmental, nutritional, pathological, and other factors. The concept of stress still eludes satisfactory definition and changes from situation to situation and from user to user. In a biological dimension stress evokes a combination of physiological and behavioural adaptations which constitute a "stress response" to aversive stimulus or stimuli which present a challenge to normal homeostasis. Unfortunately this definition is far too simple in view of the complexity of the range of potential stressors to which animals are often exposed to in the real world.

Stress can also be defined as the non-specific response of the body to any demand made upon the body (Selye, 1950). Although many workers have provided a description of the subjective term "stress", the common denominator is the lack of specificity of the conditions believed to be "stressful". During everyday function, many normal physiological challenges (e.g. exercise, nutrition) may stress an animals adaptive mechanisms. The stimulus may not be perceived as stressful by the individual, but may elicit a defined physiological response. Similarly, some stressors encountered may not trigger a physiological response, yet may be felt to be stressful in the individual. Stress is more often produced by unpleasant rather than pleasant stimuli which implicates a complex network of psycho-physiological responses, which can be grouped together in what Pasternac and Talajic (1991), call the "defence reaction" which allows the animal to face a threatening situation or run. To the ethologist, stress in an animal is defined in terms of abnormal changes in behaviour patterns. There are many difficulties involved in making decisions on an animal's stress status based on behavioural indices. Defining stress in these terms could be more comprehensive if

both physiological and behavioural indices were linked to quantify the stress status of the animal. In humans, psychological stressors can also include intense emotions such as frustration, guilt, worry, anger, resentment, grief, self-pity, inferiority complex and extreme excitement (Gray, 1982). The major integrative area of this response is the hypothalamus. When the hypothalamic defence area is stimulated, aortic blood pressure, heart rate, myocardial contractility and muscle blood flow all increase, tending to redistribute flow toward the musculoskeletal system and the cardiac muscle itself at the expense of various visceral blood flows, such as renal flow. Thus energy becomes available for fight or for flight.

The environment in which poultry are maintained is the single most important factor affecting productivity. The environment is meant to include not only the thermal environment, but refers to methods of confinement and relationship to other birds and the management system. Immediately prior to and during transportation, animals can be subjected to a wide range of stressful stimuli. These can include the initial catching, handling and loading of the animals. In transit the animals are exposed to the effects of motion, acceleration and impact many times throughout a journey, the animals must also cope with the thermal demands imposed by the transport micro-climate. It is proposed that the thermal environment is the major potential source of stress during transportation (Kettlewell, 1989), particularly in closed vehicles where the dissipation of heat and water vapour may be minimal and behavioural thermoregulation may be compromised by high stocking densities within crates. Other adverse factors encountered include withdrawal of water, restriction of behaviour, social disruption and noise. The consequences of transportation of animals even over relatively short distances can range from mild distress and aversion to physical injury and death.

If a stress syndrome appears at high ambient temperatures whether the physiological responses prevent a substantial rise in body temperature or do not, it is generally referred to as " heat stress " (Meltzer, 1984). Decrease in the performance of

chickens housed in hot and humid environments is manifested as reduced food intake, to reduce heat generated from the body metabolism, reduced growth rate and reduced food conversion efficiency. The decline in food intake and increased water intake are associated with reduced rate of body weight gain (Hurwitz *et al.*, 1980; Meltzer, 1984), a transiently raised deep-body temperature and raised respiratory rate when chickens are kept at high ambient temperatures. The birds will try to dissipate heat by a range of possible physiological and/or behavioural methods.

The poultry industry has recognized the need to house individual birds in an artificial environment to maximise productivity. Today's modern poultry structures are designed to maintain proper air movement, the necessary feed and water, and proper lighting programmes (Morris, 1994). The use of cages has resulted in a management scheme providing a micro-environment which is a key to the success of the poultry industry.

1.7.2. Heat stress

The optimal temperatures for maximum growth in the domestic chickens are between 32 and 34 degree centigrade at 1 day old, falling by about 0.5 °C/day to 19 °C at 32 days of age; the maximum body weight gain for broiler chicken takes place in the temperature range 18-24 °C (Barott and Pringle, 1949, 1950).

When exposed to high heat load, fowl, like other birds, recruit panting as a heat loss mechanism in as much as SHL is less effective. Reducing the effectiveness of panting is the by-product of additional heat produced by the intense activation of respiratory muscles for the panting process.

There have been many observations concerning the effect of the heat stress on broiler chickens and possible methods of reducing the effect of heat stress in order to improve broiler production. The interrelationship between the broiler production and the underlying physiological regulation of the growth process has also been studied.

However, little precise information is available as to the extent to which hormonal changes are related to decreased growth rate in broiler chickens exposed to high environmental temperatures. Similarly little is known of the relationship between methods used to improve the broiler production during the hot weather and hormonal balance of broiler chickens. To date no reports are available to explain the conflicting results in changes of thyroid function in heat stressed chickens.

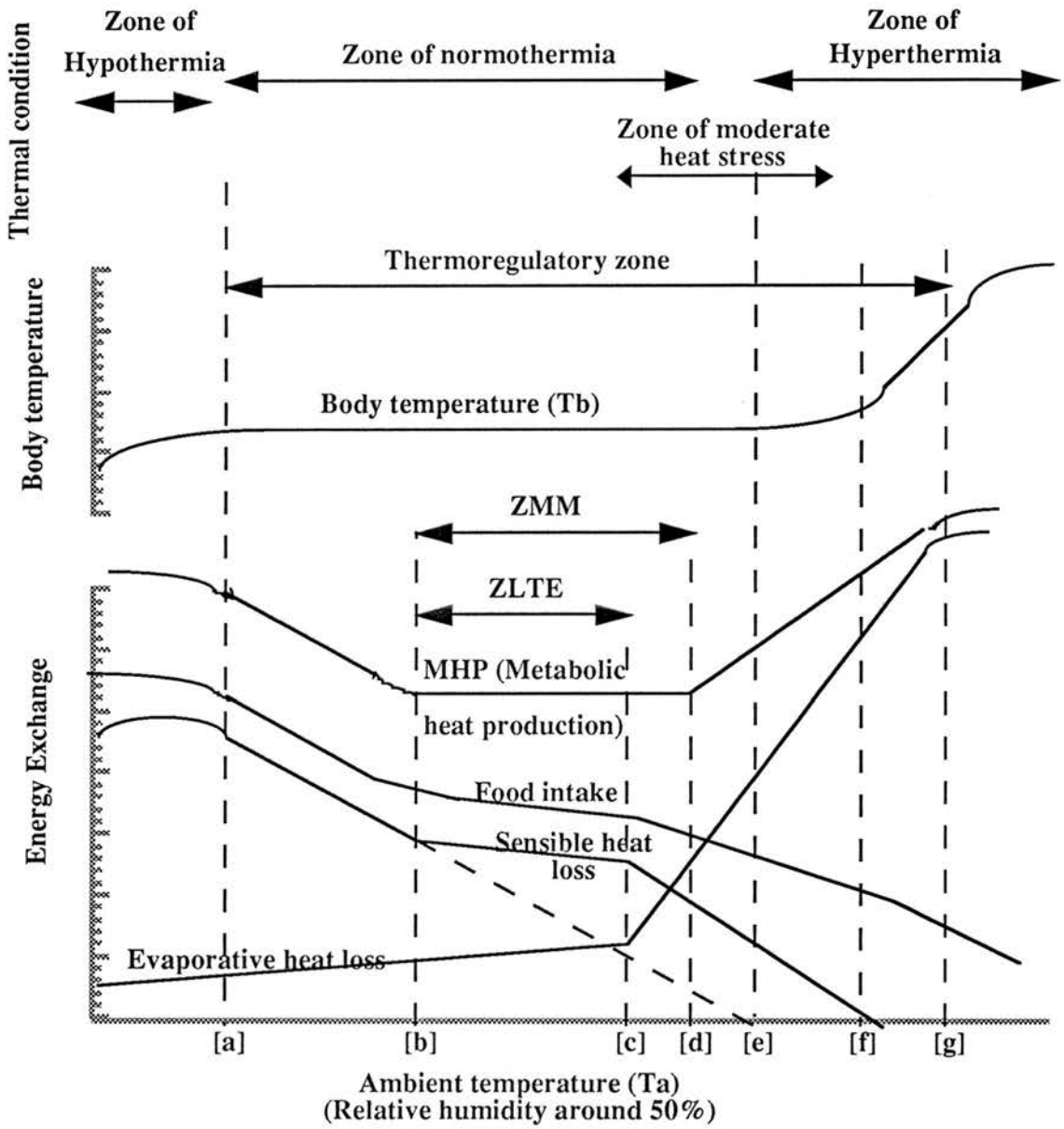
Heat stress is a physiological stress induced by ambient temperatures above the upper critical temperature of the thermoneutral zone (see Figure 5). It is the result of an imbalance between high environmental temperature, heat production and heat loss in the animal. For example if an animal can reduce its heat production and increase its heat loss under a high environmental temperature then the detrimental effects of heat stress can be reduced to a minimum (Hsia, 1990). However, this balance is easily broken. If an animal can not reduce its heat production and increase its heat loss under a high environmental temperature then the animal suffers from the severe heat stress.

1.7.3. Heat loss

The various mechanisms of heat production and heat loss cover a wide range of inter-related factors. The heat arising from the energy derived from the oxidation of food and body reserves, in addition to the ambient heat received by the animal is dissipated by radiation, convection, conduction and evaporation. These in turn are affected by the temperature, humidity and wind velocity (Hales, 1983).

The methods of dissipating heat above mentioned are themselves further influenced by the physical characteristics of the animal, such as the body surface area, type, colour and texture of the body covering, skin and the lung moisture vaporisation, water exchange as well as the thermal conductivity of the tissues peripheral to the blood flow (Hsia, 1990). All body heat produced must be dissipated either by non-evaporative channels (convection, conduction and radiation) or by

Figure 5. A model of energy exchange partitioning through a range of ambient temperatures



This generalised schematic diagram illustrates the relationship between metabolic heat production (MHP) and food or energy intake (FI) and to evaluate energy costs required for the process of growth, sensible heat loss (SHL) and evaporative heat loss (EHL) and body temperature, on the other hand, as an increase of air temperature (T_a), in a regime of free convection at 50% RH. It also illustrates the relationships between the energy flows in a generalised sense. Thus, actual values and the relationship between the curves will depend on the species of animal as well as factors of feather quality, acclimation to specific temperatures, season, nutrition status, diet, activity, age, sex, behaviour, and management systems. Adapted from Hillman *et al.* (1985).

evaporative channels (moisture vaporisation from the skin and the respiratory tract) or by both.

1.7.3.1. Non-evaporative heat loss

Under normal conditions (within the zone of thermal neutrality) the greater part of the total heat production is lost as sensible heat or by non-evaporative channels. Thus in the adult hen this constitutes about 75% of the total heat loss, although it may reach up to 90% (Freeman, 1971a). Dry heat loss is enhanced by increasing blood flow to the skin, particularly the legs and feet, and by keeping these structures and the whole body if that is possible in shade and in a flow of air. Sensible heat loss regulation within the zone of thermal neutrality consists almost exclusively of alterations in the thermal insulatory properties of the shell of the animal (feathers), alterations which themselves require the expenditure of little energy (Phillips *et al.*, 1985, Hillman *et al.*, 1985).

Above the upper critical temperature birds are still capable of some physical regulations so that the effects of the thermal loads are slightly reduced. e.g. by reducing the thermal insulatory effectiveness of the feathers, by increasing the effective surface area by standing with the neck outstretched and wings held away from the body, and by effective vasodilation in that naked parts of the body (legs, wattles and comb). So that the heat dissipated from these areas can be increased (Muiruri and Harrison, 1991).

1.7.3.2. Evaporative heat loss

Evaporative heat loss in birds is mainly carried out by the respiratory system and to a lesser extent by the skin. Evaporative heat loss plays the most important role in heat loss under high environmental temperatures. This is because the temperature difference between the body surface and the outside is small, under these circumstances the evaporative heat loss is the only avenue which does not depend on

temperature differences (Hsia, 1990).

Evaporation of water from the skin and respiratory surfaces accounts for much of the heat lost by an animal. The rate of heat dissipation depends on the surface area of the animal, the vapour pressure of the surrounding air (humidity), the rate of air movement surrounding the animal and the extent of respiratory activity (Bligh *et al.*, 1976). The importance of evaporative heat loss is manifest as the environmental temperature rises above the upper critical temperature. At lower environmental temperatures the amount of heat dissipated by the evaporation of water is relatively constant.

Despite the absence of sweat glands and the covering of the body in contour feathers, evaporative heat loss does occur across the skin in most birds that have been studied. Bernstein (1969), Van Kampen (1971) and Richards (1976b) have shown that resting birds exposed to ambient temperatures within the thermoneutral zone may evaporate 15-63% of the total evaporation via the skin. However, the amount of heat loss through this route may vary within and between species.

Marder and Ben-Asher (1983), observed that heat stressed pigeons, collared doves and palm doves have a high capacity for cutaneous evaporation. On the other hand, the partridge and quail share with many other avian species a poor capacity for cutaneous evaporation. The maximum cutaneous water lost measured by these authors in the fowl was 1.85 mg $H_2O/cm^2/hr$ at 40 °C ambient temperature.

Skin permeability and plumage thickness are two important factors influencing cutaneous water loss. Another important factor is the difference in water vapour pressure between the evaporative surface and the surrounding air, and this can be affected by the air circulation or ventilation. Such convection will remove humid air and maintain a greater vapour pressure difference across a thinner layer of boundary air. Panting and gular flutter cause the necessary convection across the respiratory

surfaces (Phillips *et al.*, 1985).

As environmental temperature rises the temperature gradient between the bird and its environment decreases with a subsequent decrease in sensible heat loss, the bird comes to rely increasingly on the evaporative heat-dissipating mechanisms in order to maintain its body temperature within the normal range. Respiratory rate increases until the bird begins to pant. Panting threshold is reached at body temperatures between 41-44 °C. The onset of panting in resting birds may be abrupt or gradually increase from the normal pattern of ventilation (Johansen and Bech, 1984; Bottje and Harrison, 1985a,b).

EHL must increase with increasing T_a to maintain thermal equilibrium because of SHL decreases. Water is lost, hence energy is lost (586 cal/g water evaporated at 20 °C), both from the skin by passive diffusion of water vapour and from the respiratory tract.

1.7.3.2.1. Cutaneous Evaporation

Evaporative water loss (EWL) occurs at the skin due to passive diffusion of water vapour through the skin. The passive diffusion component is not directly subject to thermoregulatory control. Rate of heat loss by vapour diffusion through the skin is dependent on permeance coefficient of the skin to water vapour, which is a function of air velocity, air direction, geometry, and nature of the surface, surface area of the animal, difference between saturated partial pressure of water vapour at skin temperature (T_{skin}) and partial pressure of water vapour at air temperature (T_a), and total heat of vaporisation of water.

The total heat of vaporisation is dependent on latent heat of vaporisation of water at skin temperature, difference between skin and air temperatures, and specific heat of the water vapour at constant pressure.

Typically, this diffusive component may account for a significant proportion of EHL at low T_a, but becomes a rapidly decreasing fraction at high T_a (Hillman *et al.*, 1985).

1.7.3.2.2. Respiratory Evaporation

Evaporative heat loss occurs in the respiratory process due to evaporation of water, mainly from the upper respiratory tract. In the case of a panting bird, a significant proportion of the THL is by respiratory evaporative means at high T_a. As the air passes over the wet surfaces of the respiratory tract, the air becomes saturated near body temperature (T_b), although during exhalation it is likely that some heat is lost back to the upper respiratory tract and some water vapour is condensed. However, as long as inspired air is not both saturated and equal to T_b, the expired air contains more heat and moisture than the inspired air.

The rate of respiratory EHL is dependent on mean density of air, ventilation rate of respiratory air, latent heat of vaporisation of water at skin temperature, difference between humidity ratio of expired air and humidity ratio of inspired (ambient) air, and latent heat of vaporisation of water at mean temperature of the surface of respiratory tract.

1.7.3.3. The factors known to affect evaporative heat loss

The water vapour pressure of the atmosphere surrounding a bird affects its rate of total EWL. Van Kampen (1974) measured total EHL in White Leghorn hens and found that EHL at 20 °C ambient was 13.2% of MHP at 59% RH, while at 87% RH EHL was reduced to 9.8% of MHP. At 30 °C ambient total EHL was 16.5% of MHP at 41% RH and only 3.3% of MHP at 85% RH. The effect of humidity was investigated in greater detail by Richards (1976b). Total EWL was found to have a distinct inverse linear function of water vapour pressure (within the range of 50 to 100% RH) for each T_a measured (i.e. 20, 25, 30 and 35 °C). For example, total EWL

at 20 °C was about 0.5 mg/g/hr in saturated air (100% RH), and when the air was dried to 47% RH, EWL increased threefold. Considering the profound effect that humidity has upon EWL, we should be cautious about proposing that normothermic birds generally lose a considerable portion of their total EWL from the skin if this proposal is only based on the present evidence in which dry air most commonly used during cutaneous EWL measurements.

Cutaneous EHL is higher in newly hatched quail chicks than in mature quail. In Japanese quail chicks less that 3 days old, cutaneous EHL represents about 70% MHP at 25 °C ambient. By the time the chicks reach 60 days of age or older, cutaneous EHL drops to 13% of MHP. This relative change over about 60 days reflects both a drop in cutaneous water loss (from 12.2 mg/g/hr to 3.1 mg/g/hr) and a rise in MHP (from 1.7 cc oxygen/g/hr to 2.9 cc oxygen/g/hr) at 25 °C ambient (Bernstein, 1971). Skin permeability to water in 2-day-old Japanese quail chicks decreased 43% compared to 13-day-old chicks. Within this same age period, McNabb and McNabb (1977) also measured a 42% increase in the thickness of the cornified layer of the skin, which presumably accounts for the decrease in skin permeability.

Evaporative water loss is not only influenced by ambient humidity, but is also varied by air flow and posture. Smith (1969) found that pigeons exposed to a 12-km/hr wind can tolerate (using a maximum T_b of 46 °C as a criterion) a 5.6 °C higher T_a than when exposed to a 8- km/hr wind. Smith (1969) also observed that pigeons will hold their wings out, thereby exposing the breast and under-surface of the wings to the airflow when facing upstream. Smith (1969) postulated that during flight, birds should lose more water from the skin than while at rest because the high airflow should break up the boundary layer. Recently this postulation was not substantiated, at least for ravens in flight, in which cutaneous EHL during flight was actually found to be relatively constant (about 10% of THL) through a range of T_a from 18 to 34.2 °C, while at rest at 22 °C ambient, cutaneous EHL was about the same (13% of THL) as

during flight (Hudson and Bernstein, 1981). Respiratory EHL, on the other hand, increased from 13 to 40% of MHP through the same temperature range, which was offset by a decrease in SHL. Although the raven in flight at a T_a of 34.2C did lose more heat from its cutaneous surfaces (58% of MHP) than from its respiratory surfaces, most of the heat loss from the skin was sensible, not latent. Likewise in the running domestic fowl, heat loss (sensible plus latent) from the skin exceeded respiratory EHL at 20 and 32 °C, the two Tas at which heat loss was measured (Brackenbury *et al.*, 1981).

1.7.4. Metabolic responses to heat stress

The metabolic rate of bird that is at a low ambient temperature is elevated in order to compensate for the increase in sensible heat loss, at a comfortable ambient temperature, the metabolic is at its minimal level and at a high ambient temperature the metabolic rate rises again due to activation of the cooling mechanisms.

During exposure to high thermal loads and other possible stresses or changes induced in thermoregulatory activity, the circulatory and respiratory systems, body temperature, blood chemistry and endocrine and neuroendocrine activities bring about a wide range of metabolic responses throughout the animal. These will result in alterations in the circulatory profiles of a number of metabolites and intracellular constituents including enzymes. Indeed various physiological or pathophysiological stressors are known to produce elevation in the plasma levels of intracellular enzymes.

It has frequently been observed that in mammals, both pathological and sustained physiological changes in various organ systems bring about alterations in the blood plasma levels of enzymes normally located within the intracellular and compartment (White, 1963). These enzymes are usually present in low activity in the blood plasma of the resting healthy animal. In physiological stress situations, however, an increase in the permeability of the limiting membrane may occur, thus

causing an elevation in the plasma activity of the enzymes.

Thermoregulatory behaviour involves the movement of the entire bird or one part of it, such as a limb in response to a change in either environmental or body temperature and it requires conscious effort. The most evident thermoregulatory behaviour of birds is migration to warmer or cooler areas. Some of the more obvious responses of birds to intense desert heat are to soar at altitudes where air temperature and heat are less than at ground level, or to seek shade, or to reduce activity in the hottest part of the day (Dawson and Hudson, 1970).

However these mechanisms have been lost in domestic fowl since they lost the ability to fly but, some other activities remain like keeping the wings held away from the body and elevation of the scapular feathers, both responses facilitate convective heat loss to the air. Chickens also splash water over their combs and wattles, which are cooled by evaporation of water. In cool environments chickens reduce their surface area, and hence its heat loss by hunching (Whittow, 1976).

1.7.4.1. Energetic cost of panting

A 10% rise in MHP was observed in the raven (*Corvus corax*) for each 1 °C rise in T_b as T_a exceeded T_b. This rise was calculated to be mostly due (about 70% of the total) to the increased demands of respiratory muscles and in part due (about 30% of the total) to the effect of elevated tissue temperature during hyperthermia (Marder, 1973a). During exercise in hot environments in domestic cocks, deep panting appears to account for a 12% increase in MHP over exercise alone (Brackenbury and Avery, 1980). On the other hand, an increase in the energetic cost of panting was not observed in the ostrich (*Struthio camelus*) even up to T_a as high as 52 °C, although the respiration rate did not increase above 45 to 50 breaths per minute (Crawford and Schmidt-Nielsen, 1967). In resting fowl an increase in MHP is not usually observed at T_a between 35 and 40 °C, even though the respiration rate increases substantially

from about 30 to about 150 breaths per minute (Romijn and Vreugdenhil, 1969). The true "cost of panting" requires further study. Only using changes in MHP to assess the cost of panting may not be accurate because, as is suggested for mammals, a real increase in metabolic demands by the muscles involving panting may be offset by decreased metabolic demands of other tissues, which results in little net change in overall MHP (Weathers and Schoenbaechler, 1976).

1.7.4.2. Heat acclimatisation

Acclimatisation might be defined as the long-term adaptive physiological adjustments which result in an increased tolerance to continuous or repeated exposure to complex climatic stressors produced normally under field conditions. Acclimatisation might also reflect the degree of physiological adaptation of the animal to a particular environment. The greater the extent of adaptation, the better the animal will be able to survive or to reproduce itself so that its biological characteristics may persist (Hafez, 1968).

Alterations in MHP following heat acclimation are less pronounced than are the alteration of MHP to cold acclimation (MacLeod and Mitchell, 1989). In adult fowl, the initial response to heat exposure is reduced heat production (El-Hadi and Sykes, 1980; MacLeod *et al.*, 1980a; MacLeod and Hocking 1993; MacLeod *et al.*, 1993). Fasting MHP also declines in heat-acclimated fowl (Davis *et al.*, 1972; Huston *et al.*, 1962a; Klandorf, Sharp and MacLeod, 1981), which may be due to a drop in thyroid activity (Shafie *et al.*, 1979; MacLeod and Mitchell 1989) or because body weight and egg production drop in layers (DeShazer *et al.*, 1970). Long-term adaptation to heat appears not to be due to increased efficiency of respiratory EHL (Weiss *et al.*, 1963) but rather appears to be due to enhanced SHL by increasing shell conductance by increased blood flow to the non-feathered extremities (DeShazer *et al.*, 1970).

Most modern highly productive poultry strains have been developed in

temperate countries, with little opportunity for heat tolerance to be a selection factor. Therefore when these birds are moved to tropical or subtropical countries physiological acclimatisation must provide them with protection against the heat, although their productivity may be impaired in the process.

Acclimatisation is possible in the domestic hen allowing it to survive in high ambient temperatures. Sykes and Fataftah (1986a), demonstrated that acclimatisation allowed laying hens to survive during intermittent exposure to a hot, dry climate that initially would have been lethal for them. The increased heat tolerance was reflected in the lower body temperatures, higher panting rates and decreased evaporative water loss. Strain differences in the response to heat stress were also suggested by these authors, although it was not concluded whether these are solely a reflection of body size and metabolic rate, or some other genetically determined character.

Increased body temperature is an essential prerequisite to acclimatisation, however it is not accompanied by increased heat loss. Sykes and Fataftah (1986a), reported that acclimatisation is not accompanied by an increase in evaporative heat loss, a situation opposite to that found in man and in other sweating animals.

The lower evaporative water loss is a consequence of the lower temperature of the evaporative surfaces, brought about by acclimatisation. Furthermore there is a limited scope for increasing heat loss by vasodilation since only the lower legs and the comb offer a surface for heat exchange. Thus acclimatisation is achieved by reducing heat production, rather than by increasing heat loss (Sykes and Fataftah, 1986b).

1.7.5. The factors known to affect basal metabolic rate (BMR)

1.7.5.1. Acclimation

A bird's metabolic heat production at a given ambient air temperature is strongly influenced by its thermal history prior to measurements of basal metabolic rate (Klandorf, Sharp and MacLeod, 1981). The zone of minimum metabolism (ZMM) appears to be expressed in non-acclimated birds (Which are commonly held at 20 to 25 °C for a long period prior to metabolic heat production measurements) and not in acclimated birds (which are held for a long period of time at the temperature in which metabolic heat production measurements are taken). The classical study of metabolism in fowl was carried out using non-acclimated fowl by Barott and Pringle (1946). A distinct zone of minimal metabolism was found, which varied depending on the age of these starved, restrained birds in darkness. Subsequent studies have shown a curvilinear relationship of metabolic heat production to ambient air temperature in non-acclimated birds, where at higher ambient air temperature (usually above about 20 °C) the curve flattens out into a ZMM (Van Kampen, 1974; Farrell and Swain, 1977a, b). In acclimated birds the ZMM is not expressed, instead a linear correlation of metabolic heat production to ambient air temperature is found (Romijn and Vreugdenhil, 1969; Davis *et al.,* 1973; Farrell and Swain, 1977a, b). The reason for this difference is not entirely clear. Possibly, in non-acclimated birds a distinct ZMM expresses itself because these birds utilise physical factors such as vasomotor or feather adjustments, or both, in short-term exposure to higher or lower ambient air temperature, while long-term exposure results, in a readjustment in metabolic heat production (MacLeod *et al.,* 1980a; Mitchell and MacLeod, 1983). Consistent with this hypothesis, White Leghorn hens do not show a change in metabolic heat production when moved from a room at 22 °C, in which they had been held for some

time, to a room at 28 °C ambient. However, after 3 to 12 days at 28 °C the relocated hens readjusted their metabolic heat production to a significantly lower level (15% by 12 days) than when held continuously at 22 °C (Shannon and Brown, 1969). It takes 4 weeks at 35 °C after being removed from an uncontrolled environment (7 to 18 °C until a stable, lower level of metabolic heat production is achieved; cold acclimation, on the other hand, only takes about 1 week. The presence or absence of a distinct ZMM may depend on the temperature at which a hen is a acclimated. As acclimation temperature is raised, the ZMM narrows because the upper critical temperature rises faster than the lower critical temperature. These two critical temperatures, which represent the width of the ZMM, appear to converge at an acclimation temperature of about 32 °C. Therefore, birds acclimated to ambient air temperature above 32 °C should fail to show a ZMM (Arieli *et al.*, 1980).

1.7.5.2. Age

The ZMM is widened and is shifted to a lower range of temperatures as birds mature. Measuring standard metabolic rate (SMR) in non-acclimated Rhode Island Reds, Barott and Pringle (1946) found that the ZMM ranges from about 34.5 to 36 °C, while in 1-year-old birds the zone ranges from about 17 to 24 °C. Total MHP increases as the bird matures, simply because there is an enlargement of respiratory tissue. On the other hand, in growing turkeys, as with other endotherms, the specific heat production as measured per unit of body weight decreases with age. In young Japanese quail, weight-specific SMR is lowest in newly hatched chicks and is highest in 1-week-old chicks (Blem, 1978).

1.7.5.3. Breed

In comparing SMR rates of different breeds of fowl it is important to correct for body size. When this done the differences between breeds are not so apparent. When two strains of Leghorns were compared (Warren SSL and Babcock B300) little

difference in the diurnal variation in MHP was observed. Allowing for activity and feeding of broilers and layers during metabolic measurements, broilers had a higher MHP than layers when MHP was corrected for body weight. This difference was attributed to the greater food intake and activity that broilers display compared to layers (Denbow and Kuenzel, 1981).

1.7.5.4. Feather quality

As one might expect, poorly feathered birds have a higher standard metabolic rate (SMR) than do normally feathered birds (Tullett, MacLeod and Jewitt, 1980). This is simply a response to compensate for the additional heat lost due to poor insulation (O'Neill, and Jackson, 1974b). In a White Leghorn and Australorp cross SMR was 16% greater at 8 °C ambient and 19% greater at 20 °C in poorly feathered layers than in normally feathered layers (Johnson *et al.*, 1978). The rate of increase of MHP with falling T_a below 30 °C is higher in poorly feathered birds than controls (Richards, 1977). There appears to be a threshold effect on the amount of feather loss to an increase in MHP. For example, daily MHP increases if all feathers from the breast and neck are removed, but not if feathers are removed only from the neck or only from the breast (Tullett *et al.*, 1980).

1.7.5.5. Circadian rhythm

It has been known that MHP is lower at night than during the day (Klandorf, Sharp and MacLeod, 1981). During the course of each 24 hr cycle, MHP oscillates with a regular pattern. In 1-week-old chicks, the daily difference between minimum and maximum MHP is about 24%. This difference decreases as the chicks mature, where the cyclical variation is 11% by the age of 14 weeks (Barott *et al.*, 1938). Shortening the dark period to 10 hr each day decreased the amplitude of MHP in active, feeding fowl (MacLeod *et al.*, 1980b). It was suggested that short period of darkness does not allow the minimum rate of metabolism to be realised because of a

possible carryover effect of the heat increment of feeding. It was also suggested that the difference between day and night is due to a difference in activity and food intake. Berman and Meltzer (1978) observed rhythmicity of MHP in fowl and found that MHP oscillations continued to free run. Its maintenance, however, requires that at least dim light be present to maintain the free-running rhythmicity in MHP, because its rhythmicity is extinguished in complete darkness.

1.7.5.6. Nutritional status

The heat increment of feeding apparently has a pronounced effect upon MHP in that fed chickens have a significantly higher MHP than starved hens (Farrell and Swain, 1977a, b; Lundy *et al.*, 1978; MacLeod, and Hocking, 1993). This difference increased from a low of 17% at 35 °C ambient to a high of 48% at 2 °C in acclimated broilers, while in non-acclimated broilers this difference remained at about 30% within the range of T_a from 2 to 35 °C (Farrell and Swain, 1977a, b). In this same study, food intake was about 105 g/day between 2 to 9 °C, falling at a linear rate to about 50 g/day as T_a was increased to 35 °C. The respiratory quotient (RQ) of fed fowl is about 0.96, which falls to 0.74 in starved birds (Lundy *et al.*, 1978). The RQ of fasting hens never seems to fall below 0.7 (Boshouweres and Nicaise, 1981; Geers *et al.*, 1978). Chronic glucagon infusion affected diurnal rhythms of heat production, thyroid activity and plasma metabolites (MacLeod and Mitchell, 1986).

1.7.5.7. Activity

As previously noted, activity results in a dramatic increase in MHP (Savory and MacLeod, 1980; MacLeod *et al.*, 1993). Even the simple act of standing up from a sitting position increases MHP by 40 to 45% according to direct calorimetric measurements (Deighton and Hutchinson, 1940). This estimate may be too high in that MHP measured by using indirect calorimetry reveals that standing initially increases MHP by 25% over sitting (Van Kampen, 1976a). The discrepancy between

these two studies may be due to the liberation of hot air from between the feathers when the bird stands and fluffs its feathers (Van Kampen, 1976a), or by the liberation of SHL from the feet as the bird stands, because the feet are warm when ensconced by feathers and cool immediately after exposure to cold air (Hill *et al.*, 1980). DeShazer *et al.* (1970) reported an increase between 20 and 40% in SHL when hens changed from the sitting to the standing position. After about 1 hr of standing the hen became quiet, with less head and neck movement, and the percent increase of standing over sitting dropped to 16%. In another activity which modifies MHP, Kleiber and Winchester (1933) found that huddling in 3-week-old chickens reduced standard metabolic rate (SMR) by 15% at 15 °C ambient. Van Kampen (1976a). observed that eating increased MHP over SMR by an average of 37%. This increase was shown to be due to the act of eating and not due to the heat increment of feeding. Van Kampen (1976a). estimated that about 3% of the total daily MHP of a layer can be attributed to the activity of eating.

The energetic cost of running in fowl has also been studied. Van Kampen (1976b) found that hens running on a treadmill between the speeds of 1 and 2 km/hr showed a linear increase in MHP over resting by 53 to 65%. Brackenbury and Avery (1980) also found a linear relationship of MHP to running speeds, where MHP was 11.8 times resting MHP at 9 km/ hr. Metabolic heat production was increased by an additional 12% when the cocks ran at a high T_a (30 °C) and at speeds greater than about 6.5 km/hr. This increase was probably due to the cost of deep panting observed under these conditions. In either study of running in the fowl, the zero-speed extrapolation of MHP to running speed was higher than resting MHP. This difference may be attributed to postural changes, alterations in SHL, or an increase in T_b due to running (Van Kampen, 1976b; Brackenbury and Avery, 1980).

1.7.5.8. Sex

Standard metabolic rate (SMR) of White Leghorn layers is about 50% greater per unit body weight than for cockerels (O'Neill, and Jackson, 1974a). Much of this difference is apparently due to the energetic cost of laying, because layers have a higher MHP than nonlayers (O'Neill, and Jackson, 1974b; Waring and Brown, 1967) and also because SMR in hens increases during their laying cycle (Leeson and Porter-Smith, 1970).

1.7.6. Effects of heat stress on hormones in birds

It is know that a range of stressful stimulation may increase the secretion of corticosterone from the adrenal gland, an effect mediated by release of pituitary ACTH. Administration of either ACTH or dexamethasone depresses both T4 to T3 conversion and T4 secretion in chickens (Mitchell *et al.*, 1986c). In contrast, in the chick embryo, intravenous (iv) injections of ovine corticotrophin-releasing hormone (oCRH) induced dose dependent increases in corticosterone concentration and increased concentrations of plasma T3 and T4, without affecting the T3/T4 ratio, indicating stimulation of the thyrotrophs rather than the peripheral conversion of T4 into T3 (Meeuwis *et al.*, 1989).

The major integrative area of this response is the hypothalamus. Another important element in the regulation of the stress response are the adrenal glands. Freeman (1976) has shown that the adrenal glands are central in the mediation of the stress response of the bird. Increases in the circulating level of corticosterone occur shortly after exposure to a stressor. Corticosterone is the main adrenal corticoid in the bird. Hypothalamic control of adrenal activity, through the release corticotrophin-releasing factor, appears to be important in the mediation of the stress response in the bird, also the possible extra-hypophyseal control of the adrenal is considered

important. Frankel (1970) have suggested the existence of an ACTH-like substance in the circulation of the bird.

High blood levels of corticosterone in stressed birds are considered to have some negative effects. While the stimulation of the adrenal is a necessary part of the response to stress, it has been shown that there are many side effects which have unfortunate implications to animal productivity and welfare. These may include increased susceptibility to viral diseases, impaired immunological reactivity and reduced productivity in terms of growth and egg production (Freeman, 1976).

Heat stress is a condition in which the body temperature is so high as to interfere with normal homeostatic processes and if continued can lead to prostration and death. Heat stress is the result of an imbalance between high environmental temperature, heat production and heat loss in the animal. For example if an animal can reduce its heat production and increase its heat loss under a moderately high environmental temperature then the detrimental effects of heat stress can be reduced to a minimum (Hsia, 1990). However this is not the normal pattern in tropical regions where the animal has to cope with high environmental temperatures and therefore this balance is easily broken.

1.7.6.1. Effects of heat stress on thyroid hormones in birds

Early investigators reported that the thyroid weight of chickens and pigeons is greater in the winter than in the summer (Figure 7). Histologic changes indicating follicular stimulation within the thyroid coincide with the onset of cold weather (Hohn, 1949). However, that study also showed greater thyroid weight in the summer (July) than in the cold months. It has also been reported that the thyroid gland decreases in size and activity when birds become acclimated to high environment temperatures (Dale and Fuller, 1980).

Figure 7. Decreased thyroid weight (percentage of control) during heat stress

Species and Sex	Treatment & Environment	Thyroid Wt. (%)	Authors
Statistically significant, $p<0.05$			
Male White Plymouth Rock aged 6-month	August	46	Reineke and Turner (1945).
Male adult White Plymouth Rock	Kept at 32.2°C since hatch	49	Huston and Carmon (1962b)
Female Broiler Chicks aged 1-4 wks	at 36°C for 10 hr/day for 3 weeks	56	Kutlu and Forbes (1993b).
Female adult White Plymouth Rock	Kept at 32.2°C since hatch	58	Huston and Carmon (1962b)
Male White Plymouth Rock aged 12 wks	Kept at 31.1°C since hatch	66	Huston *et al.* (1962c)
Female adult New Hampshire	Kept at 32.2°C since hatch	72	Huston and Carmon (1962b)
Female Leghorn hens aged 450-day	29.44°C, 70% RH for 174-day	76	Mueller and Amezcus (1959).
Female Leghorn aged 56-70 days	37 °C for 16 days	77	Cogburn and Harrison (1980).
Female Leghorn aged 56-70 days	37 °C for 16 days	78	Cogburn and Harrison (1980).
Non-significant			
Male adult New Hampshire	Kept at 32.2°C since hatch	51	Huston and Carmon (1962b)
Female Plymouth Rock aged 6-month	August	63	Reineke and Turner (1945).
Female adult White Leghorn	Kept at 32.2°C since hatch	70	Huston and Carmon (1962b)
Male adult White Leghorn	Kept at 32.2°C since hatch	83	Huston and Carmon (1962b)
Female Japanese quail aged 6-7-week	34 °C, 89% RH for 1-hour	90	Mueller and Amezcus (1959).
Female Leghorn hens aged 450-day	29.44°C, 25% RH for 174-day	90	Mueller and Amezcus (1959).
Female Leghorn aged 56-70 days	Photophase 37 °C for 16 days	95	Cogburn and Harrison (1980).

An increase in the size of the thyroid may be caused by an increase in cell size (hypertrophy) and/or an increase in the number of cells (hyperplasia), associated with a reduction in colloid content of the follicles. Enlarged thyroids may reflect either a hyper- or hypo-functioning gland. When exposed to high ambient temperature, the birds are able to reduce their basal metabolic rate (which should result in a decrease in internal heat production). Since thyroxine has a major influence on basal metabolic rate, this would indicate a greater reduction in thyroid activity.

The response of the thyroid to either high or low ambient temperature might be expected to consist of two responses, an acute one and a long term one. Generally, the endocrine system responds more slowly to environmental changes than the nervous system. Based on survey studies and on the basis of studies in which T3 and T4 were administered, it appears that T3 serves an important function in the regulation of metabolic heat production and temperature regulation, especially when long term adjustments to exposure to low or high ambient temperature are considered. T3 also appears to be a more potent hormone than T4 in stimulating metabolism. The stimulation of metabolism probably occurs through the enhancement of oxidative phosphorylation by thyroid hormones in the mitochondria oxidative mechanism (Sterling *et al.*, 1977).

According to Klandorf *et al.* (1981), there is a higher correlation between plasma T3 concentration and metabolic heat production, than between plasma T4 concentration and metabolic heat production. Figure 8 showed that high environmental temperatures decreased the amount of circulating T4 in chickens exposed to 32.2 °C and above this temperature for either short or long term. Figure 8 also showed that high environmental temperatures increased the amount of circulating T4 in chickens exposed to over 30 °C for either short or long term. In contrast, De-Andrade *et al.* (1977) reported that the level of T4 was found to be a little higher in cyclic high environment temperature than in constant high ambient temperature.

The physiological importance of the regulation of thyroid output by environmental temperature is presumably through the influence of thyroid hormones on the metabolic rate. In mammals the direct relationship between the thyroid activity and basal metabolic rate (BMR) has been well established. Increasing environmental temperatures have been shown to result in reduction of metabolic rate in mammals, which closely corresponds to the observed reduction in the thyroid activity. Similar responses have been observed in birds although there is little information about the subject (Falconer, 1971).

It seems generally accepted that the effect of high ambient temperatures on the avian thyroid gland is a reduction in its secretion rate. This effect seems well established and has been reported by many authors. It has, however, been demonstrated, on a number of occasions, that the changes of thyroid hormones in birds exposed to heat stress, are not consistent. In Figures 8 and 9 the findings from 15 papers are reported which indicate conflicting results in relation to the changes of thyroid hormones in birds exposed to heat stress. This section considers the factors which might cause the conflicting results.

The depressed growth rate in chickens exposed to heat stress conditions may result in changing levels of both T4 and T3, although the exact mechanisms controlling growth rate of chronically heat stressed chickens are poorly understood. However, the existing literature on the effects of heat stress on thyroid hormones is very confusing (Figures 8 and 9). In the domestic fowl, an exposure to high ambient temperature may result in increased (Bowen and Washburn, 1985; Cogburn and Harrison, 1980; Iqbal *et al.*, 1987; Moss and Balnave, 1978) or decreased (Bobek *et al.*, 1980; May, 1978; May and McNaughton, 1980; Rudas and Pethes, 1984) or unchanged (Bowen and Washburn, 1985; Cogburn and Harrison, 1980; Klandorf *et al.*, 1981; May, 1978; May and McNaughton, 1980; Moss and Balnave, 1978) plasma concentration of T4. Similarly plasma T3 may decrease (Bobek *et al.*, 1980; Cogburn

and Harrison, 1980; Iqbal *et al.*, 1987; Klandorf *et al.*, 1981; May, 1978; May and McNaughton, 1980; May *et al.*, 1986), increase (Bobek *et al.*, 1980; Bowen and Washburn, 1985) or remain unchanged (Bobek *et al.*, 1980; Bowen and Washburn, 1985; Klandorf *et al.*, 1981; May, 1978; May *et al.*, 1986; Rudas and Pethes, 1984) (Figures 8 and 9). McFarland *et al.* (1966) reported that the calculated half-life of T4 for Japanese quail kept at 32 °C was 36% lower than for those maintained at 21 °C.

Inspection of the 15 different studies analysed and compared in table 8 and 9 indicates that many different magnitudes and durations of heat stress have been employed by the various groups. In addition birds of different ages and sex have been examined in each study. It is thus perhaps not surprising that no single integrated, unifying model of the thyroid hormone response to heat stress in poultry has been established. Indeed it might be proposed that no such simple model might adequately describe the interaction of the many components of this complex system over a wide range of thermal environments.

Iqbal *et al.* (1987) reported that changes in circulating T3 and T4 concentrations upon a prolonged warm treatment (37 degrees C) of young chickens fed *ad libitum* resulted in significantly lower T3 and higher T4 concentrations. After a prolonged hot treatment, the T4 or T3 responses to TRH were abolished.

Exposure of quail to 34 °C - 35 °C decreased plasma T4 concentrations. T3 concentration fluctuated over a narrow range with no clear trend, although oxygen consumption was reduced by 48 h of exposure to warm temperature (Bobek *et al.*, 1980). Moss and Balnave (1978) measured plasma thyroxine and thyroid weight in chicks kept at 22 °C and 30 °C for 28 days. They observed a progressive increase in T4 from 8.0 ng/ml to 17.5 ng/ml in birds at 30 °C compared to 8.0 ng/ml to 8.6 ng/ml in birds at 22 °C. This was associated with a 30% reduction in thyroid weight from 52 to 36 mg/kg body weight at the higher temperature.

Conflicting results from different studies suggest that the response of the thyroid to high ambient temperatures might consist of two responses, an acute one and a long term one. It appears that T3 serves an important function in the regulation of metabolic heat production and temperature regulation, especially when long term adjustments to exposure to high ambient temperatures are considered. T3 also appears to be a more potent hormone than T4 in stimulating metabolism (Sterling *et al.*, 1977).

These conflicting results may also be associated with the larger variability of performance in birds above 32 °C discussed in Section 1.8 ((see Figures 10 and 11, following page 94 for details). The larger variability of performance in birds above 32 °C might be a consequence of the interaction of other factors (e.g. humidity, nutritional status, diet composition, stocking density, activity, acclimation, strain, sex, feather quality, or air movement), which may cause these conflicting results of the thyroid function response to high ambient temperatures. These factors should, therefore, be considered and controlled in future experimental designs to minimise the risk of un-repeatable results.

1.7.6.2. Seasonal changes in thyroid function

While the commercial poultry producer endeavours to standardise the environment as far as possible, it is of interest to observe the natural seasonal fluctuations of thyroid activity in a feral population of birds. In Ruffed grouse (*Bonasa umbellus*), both T4 and T3 concentrations in the plasma were least during the cold winter months and increased sharply at the onset of gonadal development in early spring. The peak concentrations occurred at about the time of egg laying and incubation, and T3 in particular showed a fall during the brooding-moulting period in mid- to late-summer (Garbutt *et al.*, 1979). The spotted munia (a tropical finch) shows a seasonal cyclicity in thyroid function, the lowest concentration of thyroid hormones occurring in late summer. The apparent difference between these data and those for chicks exposed to cold may reflect the slow physiological adaptation to cold in a

Figure 8. Changes in T4 (percentage of control)

Species and Sex	Age	Treatment & Environment	T4 (%)	Authors
Positive response (statistically significant, p<0.05)				
Short term heat stress:				
Male Broilers	28 days	Handling and Heating at 50°C	122	Bowen and Washburn (1985).
Mixed Japanese quail	28 days	Heating for 30 min at 50°C	133	Bowen and Washburn (1985).
Mixed Japanese quail	28 days	Heating for 30 min at 50°C	136	Bowen and Washburn (1985).
Mixed Japanese quail	28 days	Heating for 30 min at 50°C	142	Bowen and Washburn (1985).
Long term heat stress:				
Male White Leghorn	56-70 days	37 °C for 16 days	107	Cogburn and Harrison (1980).
Female White Leghorn	56-70 days	Photophase 37 °C for 16 days	129	Cogburn and Harrison (1980).
Male Unknown breeds	25-days	at 30°C for 20 days	152	Moss and Balnave (1978)
Male Unknown breeds	18-days	at 30°C for 16 days	156	Moss and Balnave (1978)
Mixed Hisex White chicks	3-week	38 °C, 50-60% RH for 7 days	169	Iqbal *et al.*, 1987
Male Unknown breeds	25-days	at 30°C for 28 days	203	Moss and Balnave (1978)
Negative response (statistically significant, p<0.05)				
Short term heat stress:				
Male Unknown breeds	5 weeks	acute heat (35 °C) stress for 60 minutes	56	Rudas and Pethes (1984)
Female Japanese quails	6-7 weeks	34°C, 89%RH for 24 hr	58	Bobek *et al.* (1980).
Female Japanese quails	6-7 weeks	34°C, 89%RH for 48 hr	56	Bobek *et al.* (1980).
Female Japanese quails	6-7 weeks	34°C, 89%RH for 12 hr	75	Bobek *et al.* (1980).
Female Japanese quails	6-7 weeks	34°C, 89%RH for 5 hr	81	Bobek *et al.* (1980).
Long term heat stress:				
Female Broiler chicks	49-50 days	32.2 °C for 50 days at 8:00	57	May (1978).
Female Broiler chicks	34-37 days	32.2 °C for 35 days (day 1)	72	May and McNaughton (1980).
No response (non-significant) trend				
Short term heat stress:				
Male Broilers	15-28 days	0.2% thiouracil & heating at 50°C	91	Bowen and Washburn (1985).
Female Japanese quails	6-7 weeks	34°C, 89%RH for 1 hr	92	Bobek *et al.* (1980).
Mixed Japanese quail	28 days	Heating for 30 min at 50°C	100	Bowen and Washburn (1985).
Male Broilers	28 days	Heating for 1 hr at 50°C	119	Bowen and Washburn (1985).
Male Broilers	28 days	Heating for 1 hr at 50°C	129	Bowen and Washburn (1985).
Long term heat stress:				
Female Broiler chicks	49-50 days	32.2 °C for 50 days at 10:00	76	May (1978).
Female White Leghorn	22+14=36 weeks	32 °C for 10-day	80	Klandorf *et al.* (1981).
Female Broiler chicks	49-50 days	32.2 °C for 50 days at 12:00	81	May (1978).
Female Broiler chicks	34-37 days	32.2 °C for 35 days (day 1)	84	May and McNaughton (1980).
Female Broiler chicks	34-37 days	32.2 °C for 36 days (day 2)	85	May and McNaughton (1980).
Female Broiler chicks	34-37 days	32.2 °C for 37 days (day 3)	88	May and McNaughton (1980).
Female Broiler chicks	41 day	Acclimated (at 24, 35, 24 °C for 3 days)	88	May *et al.* (1986).
Female White Leghorn	22+14=36 weeks	32 °C for 3-day	88	Klandorf *et al.* (1981).
Female Broiler chicks	49-50 days	32.2 °C for 50 days at 12:00	91	May (1978).
Female Broiler chicks	34-37 days	32.2 °C for 37 days (day 3)	94	May and McNaughton (1980).
Female Broiler chicks	49-50 days	32.2 °C for 50 days at 8:00	95	May (1978).
Female Broiler chicks	49-50 days	32.2 °C for 50 days at 14:00	96	May (1978).
Female White Leghorn	22+14=36 weeks	32 °C for 1-day	97	Klandorf *et al.* (1981).
Female Broiler chicks	34-37 days	32.2 °C for 36 days (day 2)	99	May and McNaughton (1980).
Male Unknown breeds	18-days	at 30°C for 2 days	100	Moss and Balnave (1978)
Female Broiler chicks	49-50 days	32.2 °C for 50 days at 14:00	102	May (1978).
Female White Leghorn	22+14=36 weeks	32 °C for 7-day	103	Klandorf *et al.* (1981).
Female Broiler chicks	49-50 days	32.2 °C for 50 days at 12:00	104	May (1978).
Female White Leghorn	56-70 days	Scotophase 37 °C for 16 days	104	Cogburn and Harrison (1980).
Female White Leghorn	56-70 days	37 °C for 16 days	108	Cogburn and Harrison (1980).
Male Unknown breeds	18-days	at 30°C for 6 days	109	Moss and Balnave (1978)
Female White Leghorn	56-70 days	37 °C for 16 days	110	Cogburn and Harrison (1980).
Mixed Hisex White chicks	3-week	38 °C, 50-60% RH for 7 days	110	Iqbal *et al.*, 1987
Male Broilers	22 day	Acclimated (at 24, 35, 24 °C for 3 days)	111	May *et al.* (1986).
Male Unknown breeds	25-days	at 30°C for 6 days	112	Moss and Balnave (1978)
Female White Leghorn	56-70 days	Scotophase 37 °C for 16 days	113	Cogburn and Harrison (1980).
Female White Leghorn	56-70 days	37 °C for 16 days	114	Cogburn and Harrison (1980).
Female Broiler chicks	49-50 days	32.2 °C for 50 days at 14:00	115	May (1978).
Female Broiler chicks	41 day	Acclimated (at 24, 35, 24 °C for 3 days)	117	May *et al.* (1986).
Female Broiler chicks	49-50 days	32.2 °C for 50 days at 10:00	121	May (1978).
Female Broiler chicks	49-50 days	32.2 °C for 50 days at 10:00	123	May (1978).

natural environment, in which hormone utilization may rise considerably, together with increased secretion, while plasma concentrations actually fall.

In most species of wild birds thus far studied there is a relationship between thyroidal and gonadal functions. Physiological and morphological data have been used to illustrate this relationship, but the precise nature of this relationship differs between species. Seasonal changes in extrathyroidal conversion of T4 to T3 may alter with seasonal reproduction (Pathak and Chandola, 1982; Cogburn and Freeman, 1984). In any measure of seasonal effects, it is necessary to examine changes in temperature, light, food intake, reproductive cycle, and molt, which vary with the season. The timing of the decrease in thyroid activity is associated with the onset of gonadal recrudescence and factors such as food and temperature may be regulating this cycle of development. Alternatively in ducks and teal it is changes in day-length which determine the breeding period. The annual increase in plasma concentrations of T4 is associated with a marked decline in gonadal function. In general the studies of endocrine cycles in thyroidectomized or gonadectomised birds show a close interaction between the thyroid and sexual cycles which tend to be inversely related.

1.7.7. Other endocrine influences on thyroid hormones

It has been suggested that the thyroid hormones, especially T3, are most important in the long-term adaptation to temperatures outside ZMM, while corticosteroids and catecholamines are more important in the immediate adjustment to hot or cold. Glucagon, by its metabolic effect and not by any thermogenic effect, aids in adapting to low T_a (Hillman *et al.*, 1985). Whilst insulin, glucagon and corticosterone may play direct or indirect roles in the regulation of thyroid hormone secretion and metabolism in the domestic fowl (Mitchell *et al.*, 1986c; Mitchell and Raza, 1986a).

Figure 9. Changes in T3 (percentage of control)

Species and Sex	Age	Treatment & Environment	T3(%)	Authors
Positive response (statistically significant, $p<0.05$)				
Short term heat stress:				
Female Japanese quail	6-7 weeks	34°C, 89%RH for 5 hr	155	Bobek *et al.* (1980).
Male Broiler Chicks	28 days	Heating for 1 hr at 50°C	156	Bowen and Washburn (1985).
Mixed Japanese quail	28 days	Heating for 30 min at 50°C	158	Bowen and Washburn (1985).
Mixed Japanese quail	28 days	Heating for 30 min at 50°C	171	Bowen and Washburn (1985).
Male Broiler Chicks	28 days	Handling and Heating at 50°C	173	Bowen and Washburn (1985).
Negative response (statistically significant, $p<0.05$)				
Short term heat stress:				
Female Leghorn	36 weeks	32 °C for 1-day	58	Klandorf *et al.* (1981).
Male Broiler chicks	49 day	35 for 3 d & at 41 °C for 3 hr	84	May *et al.* (1986).
Long term heat stress:				
Mixed Hisex White chicks	3-week	38 °C, 50-60% RH for 7 days	20	Iqbal *et al.*, 1987
Mixed Hisex White chicks	3-week	38 °C, 50-60% RH for 7 days	20	Iqbal *et al.*, 1987
Female Leghorn Light off for 10 hr	36 weeks	32 °C for 14-day at 04:00	37	Klandorf *et al.* (1981).
Female Leghorn Light off for 10 hr	36 weeks	32 °C for 13-day at 04:00	38	Klandorf *et al.* (1981).
Female Broiler chicks	34-37 days	32.2 °C for 35 days (day 1)	49	May and McNaughton (1980).
Female Broiler chicks	49-50 days	32.2 °C for 50 days at 12:00	52	May (1978).
Female Leghorn Light on for 4 hr	36 weeks	32 °C for 13-day at 08:00	53	Klandorf *et al.* (1981).
Male Leghorn	56-70 days	Scotophase 37 °C for 16 days	54	Cogburn and Harrison (1980).
Male Leghorn	56-70 days	Scotophase 37 °C for 16 days	56	Cogburn and Harrison (1980).
Female Leghorn	56-70 days	37 °C for 16 days	59	Cogburn and Harrison (1980).
Female Leghorn	56-70 days	37 °C for 16 days	64	Cogburn and Harrison (1980).
Female Broiler chicks	49-50 days	32.2 °C for 50 days at 8:00	66	May (1978).
Female Broiler chicks	49-50 days	32.2 °C for 50 days at 10:00	66	May (1978).
Female Leghorn	56-70 days	37 °C for 16 days	67	Cogburn and Harrison (1980).
Female Leghorn	56-70 days	Photophase 37 °C for 16 days	67	Cogburn and Harrison (1980).
Male Leghorn	56-70 days	Photophase 37 °C for 16 days	67	Cogburn and Harrison (1980).
Female Leghorn	36 weeks	32 °C for 10-day	67	Klandorf *et al.* (1981).
Female Broiler chicks	49-50 days	32.2 °C for 50 days at 14:00	67	May (1978).
Female Broiler chicks	34-37 days	32.2 °C for 36 days (day 2)	69	May and McNaughton (1980).
Female Broiler chicks fed 0.3% aspirin	34-37 days	32.2 °C for 36 days (day 2)	69	May and McNaughton (1980).
Female Broiler chicks fasting for 0.5 hr	49-50 days	32.2 °C for 50 days at 10:00	70	May (1978).
Female Leghorn Light on for 8 hr	36 weeks	32 °C for 13-day at 12:00	72	Klandorf *et al.* (1981).
Female Leghorn	36 weeks	32 °C for 3-day	75	Klandorf *et al.* (1981).
Female Broiler chicks	34-37 days	32.2 °C for 37 days (day 3)	78	May and McNaughton (1980).
Female Broiler chicks fed 0.3% aspirin	34-37 days	32.2 °C for 37 days (day 3)	78	May and McNaughton (1980).
Female Leghorn Light on for 12 hr	36 weeks	32 °C for 13-day at 16:00	79	Klandorf *et al.* (1981).
Female Broiler chicks fasting for 4.5 hr	49-50 days	32.2 °C for 50 days at 14:00	81	May (1978).
Female Japanese quail	6-7 weeks	34°C, 89%RH for 48 hr	82	Bobek *et al.* (1980).
No response (non-significant) trend				
Short term heat stress:				
Female Broiler chicks	41 day	at 24, 35, 24 °C for 3 days	94	May *et al.* (1986).
Mixed Japanese quail	28 days	Heating for 30 min at 50°C	97	Bowen and Washburn (1985).
Female Broiler chicks	41 day	at 24, 35, 24 °C for 3 days	99	May *et al.* (1986).
Male Broiler chicks	22 day	at 24, 35, 24 °C for 3 days	100	May *et al.* (1986).
Male Unknown breed Chicks	5 weeks	35 °C for 60 minutes	103	Rudas and Pethes (1984)
Male Broiler Chicks	15-28 days	0.2% thiouracil & heating at 50°C	104	Bowen and Washburn (1985).
Female Japanese quail	6-7 weeks	34°C, 89%RH for 1 hr	107	Bobek *et al.* (1980).
Female Japanese quail	6-7 weeks	34°C, 89%RH for 12 hr	111	Bobek *et al.* (1980).
Male Broiler Chicks	28 days	Heating for 1 hr at 50°C	115	Bowen and Washburn (1985).
Female Japanese quail	6-7 weeks	34°C, 89%RH for 24 hr	120	Bobek *et al.* (1980).
Long term heat stress:				
Female Leghorn Fasting for 28 hr	36 weeks	32 °C for 13-day at 08:00	78	Klandorf *et al.* (1981).
Female Broiler chicks fed 0.3% aspirin	34-37 days	32.2 °C for 35 days (day 1)	83	May and McNaughton (1980).
Female Leghorn Fasting for 32 hr	36 weeks	32 °C for 13-day at 12:00	83	Klandorf *et al.* (1981).
Female Leghorn	36 weeks	32 °C for 7-day	88	Klandorf *et al.* (1981).
Female Leghorn Fasting for 36 hr	36 weeks	32 °C for 13-day at 16:00	94	Klandorf *et al.* (1981).
Female Leghorn Fasting for 52 hr	36 weeks	32 °C for 14-day at 0400	98	Klandorf *et al.* (1981).
The effect of refed after 24-hours' fasting in female broilers exposed at 32.2 °C for 50 days:				
Female Broiler chicks fasting for 18 hr	49-50 days	32.2 °C for 50 days at 10:00	78	May (1978).
Female Broiler chicks fasting for 16 hr	49-50 days	32.2 °C for 50 days at 8:00	84	May (1978).
Female Broilers 2.5hr after refed from 24hr's fasting	49-50 days	32.2 °C for 50 days at 12:00	111	May (1978).

1.7.7.1. Adrenal hormones

The avian suprarenal or adrenal glands consist of interrenal and chromaffin tissues which are intermingled in a cortex and medulla as in mammalian adrenals. The interrenal tissue is homologous with the mammalian adrenal cortex; the chromaffin tissue is homologous to the mammalian adrenal medulla. The interrenal glands of chickens and other poultry secrete mainly corticosterone and aldosterone (Ringer, 1970; Assenmacher, 1973). The secretion of corticosterone is mainly under the control of the anterior pituitary through the secretion of adrenocorticotrophic hormone (ACTH).

1.7.7.1.1. Adrenal cortex hormones

Plasma corticosterone level, which is considered as an acute stress indicator, was increased in broiler chickens in response to acute heat stress (Edens and Siegel, 1975), while chronic high environmental temperatures decreased Plasma corticosterone level (Beuving and Vonder 1978; Iqbal *et al.,* 1990). A possible explanation may be the difference in methodology of blood sampling. The lower plasma corticosterone levels in birds exposed to chronic high ambient temperature might not occur if obtaining blood via a catheter implanted in the wing vein without handling. Johnson (1981) has shown that such different sampling methods affect the corticosterone concentrations in plasma.

The response of the interrenal glands to exposure to cold or hot environments is less specific than response of the thyroid because the adrenal response is a general response to stress. One would, therefore, expect an increase in corticosterone concentrations in response to sudden cold or heat stress, as indeed has been observed. It should be noted that in pigeons, intravenous corticosterone injections do not affect body temperature (T_b) at either 6, 25, or 35 °C (Hissa *et al.*, 1980).

Injections of ACTH (20 IU kg^{-1}) or administration of dexamethasone (1 mg kg^{-1}) decreased plasma thyroid hormone levels and heat production in the domestic fowl (Mitchell *et al.*, 1986c). These findings indicated that corticosterone may decrease peripheral 5'-monodeiodination of T4 to T3. In contrast, in the chick embryo, intravenous (iv) injections of ovine corticotrophin-releasing hormone (oCRH) induced dose dependent increases in corticosterone concentration and increased concentrations of plasma T3 and T4, without affecting the T3/T4 ratio, indicating stimulation of the thyrotrophs rather than the peripheral conversion of T4 into T3 (Meeuwis *et al.*, 1989).

1.7.7.1.2. Adrenal medulla hormones

The principal hormones secreted by the chromaffin tissue are the catecholamines: epinephrine (E - or adrenaline) and norepinephrine (NE - or nor-adrenaline). The secretion of these hormones in response to a stressful stimulus is usually immediate because the chromaffin tissue is innervated by the sympathetic nervous system, and thus the time constant of the response is closer to that of the nervous system than to that of the endocrine system, as discussed for the thyroid and interrenal tissue. There is an increased release of catecholamine during the initial response to either cold or heat, but during continuous exposure to hot or cold environments, the secretion of adrenaline and noradrenaline returns to basal levels. Intramuscular injections of norepinephrine in pigeons acclimated either to T_a = 30 °C or to T_a = 2 °C and tested at T_a = 6 °C, resulted in (1) a decrease in body temperature in both warm-acclimated and cold-acclimated birds, (2) a decrease in O_2 consumption in warm-acclimated but not in cold-acclimated birds, and (3) inhibition of shivering and vasoconstriction in the warm-acclimated but not in cold-acclimated pigeons. When warm-acclimated and cold-acclimated pigeons were tested at T_a = 32 °C the administration of norepinephrine was followed by an increase in O_2 consumption, hyperthermia, and no effect on vasomotion of the foot; under these conditions the

cold-acclimated birds showed a greater response than the warm-acclimated ones (Hissa *et al.*, 1975).

As might be expected, the thyroidal status of the birds affects the response to norepinephrine. In hyperthyroid pigeons (75 μg T4 per kilogram body weight every 2 days for 3 weeks), norepinephrine induced hyperthermia at T_a = 22 °C, whereas at T_a = 6 °C NE induced hyperthermia which was more marked than in thiouracil-treated hypothyroid pigeons (with no controls being used) (Saarela and Hissa *et al.*, 1977).

In animals under heat stress for a long time the depression in the adrenal cortex activity may be a protective adjustment by the animal to depress its heat production. Thus low cortisol concentrations in plasma may reflect adaptation to a hot climate. The hormones in the adrenal medulla are also involved in birds' heat stress responses. The immediate increase in catecholamine levels in blood in response to a stressor in chickens is reflected by peripheral vasoconstriction, increased heart rate and increases in plasma glucose. It also has been demonstrated directly by measurement of the hormones in the plasma in both chickens and turkeys (Freeman, 1976).

1.7.7.2. Pancreatic hormones

1.7.7.2.1. Glucagon

Glucagon is a protein hormone secreted by the pancreas and the intestines. Its main effects are stimulation of glycogenolysis in liver and muscle and lipolysis of adipose tissue, thus mobilising glucose and fatty acids. The hormone is secreted in response to stress such as handling, and exposure to cold or hot environments (Hillman *et al.*, 1985). The glucagon response to stress is not specific. Although glucagon is not thermogenic, it may have an important adaptive function in thermoregulation by its metabolic effects (Freeman, 1975).

Injections of glucagon (50 μg kg^{-1}) produced a biphasic response in plasma T4

level in both fed and fasted chickens. Injections of glucagon decreased plasma T3 levels in the domestic fowl possibly as a consequence of inhibition of peripheral 5'-monodeiodination (Mitchell and Raza, 1986a).

1.7.7.2.2. Insulin

Insulin is a protein hormone secreted by the pancreas and plays a key role in the metabolic distribution of ingested nutrients. Its main effects are stimulation of glycogenesis in liver and muscle, and thus reduces blood glucose concentration. High concentration of blood glucose induces insulin release. Insulin has a key role in regulating hepatic lipogenesis in mammals, but its function is not quite clear in birds (Griffin, 1993). Lipogenesis in liver slices and hepatocytes in only slightly elevated by short term incubation with insulin (Vasilatos-Younken, 1986).

Insulin injection gave stimulated peripheral monodeiodination as reflected by a transient increase in plasma T3 in birds (Mitchell and Raza, 1986a). This may have resulted from an increased uptake of glucose by hepatocytes (Unger, 1983) which would stimulate T4 to T3 conversion (Burman *et al.*, 1979). The subsequent large decreases in available glucose might then be responsible for the extended inhibition of T3 production or plasma T3 level (Burman *et al.*, 1979). Alternatively this may be the result of the reduced concentration of circulating T4. The role of glucose in the regulation of T3 production in chickens is not clear.

Secretion of glucagon and insulin may exhibit a reciprocal relationship (Cramb and Langslow, 1984) mediated through changes in plasma glucose. It has been demonstrated that inadequate glucose delivery inhibits pituitary thyrotrophic function (Burman *et al.*, 1980; Rojdmark and Nygren, 1983) and consequently would reduce T4 secretion (Mitchell and Raza, 1986a). Both pancreatic hormones inhibit secretion of growth hormone (Foltzer *et al.*, 1981) which is reported to reverse the fasting induced decrease in plasma T3 in chickens (Kühn *et al.*, 1985; Mitchell and Raza,

1986a). Glucagon injection (Okuno *et al.*, 1982) and insulin injection (Viega *et al.*, 1983) have been reported to stimulate adrenocortical activity which may inhibit both thyroidal secretion (Mitchell and Raza, 1986a; Pamenter and Hedge, 1980) and peripheral monodeiodination (Decuypere *et al.*, 1983). Glucagon also stimulates release of catecholamines from the adrenal medulla (Foa, 1973) which are known to influence thyroid hormone metabolism (Rothwell *et al.*, 1982). The effects of glucagon and insulin upon plasma T4 and T3 concentrations may be mediated or influenced by a number of other recognised responses to administration of glucagon or insulin (Mitchell and Raza, 1986a). These actions may be attributable to direct or indirect secondary mechanisms and it is suggested that the pancreatic hormones which are central in the control of carbohydrate and lipid metabolism may also have a role in the regulation of thyroid hormone activity and therefore energy expenditure in the domestic fowl (Mitchell and Raza, 1986a).

1.7.7.3. Gonadal hormones

Luteinizing hormone, which is the ovulation-inducing hormone in the chicken, has an indirect effect on T_b because it causes the rupture of the follicle which, in turn, influences the rise in temperature usually associated with oviposition, but not requiring oviposition for the rise in T_b (Klandorf *et al.*, 1982).

Prolactin may have an effect on T_b regulation by an effect on the peripheral conversion of T4 to T3. In laying and in incubating bantams, injection of (mammalian) prolactin resulted in an increase in the concentration of T3 in the plasma without affecting the T4 concentration. This suggests that the prolactin may have had its effect through an influence on the peripheral metabolism of T4 to T3 (Klandorf *et al.*, 1982). The adaptive advantage of such an effect of prolactin would be to stimulate MHP via the thyroid at a time that the birds are not eating much, i.e., during incubation of the eggs, a time when it is crucial that T_b be maintained to warm the eggs (Klandorf *et al.*, 1982).

It appears that gonadal hormones have little if any effect on the body temperature of chickens except for the ruptured follicle hormones. The interaction between the gonads and the thyroid of birds, however, is more pronounced than in mammals, where castration causes only slight effects on thyroid activity. Castration stimulates pituitary thyrotrophs in the duck (Tixier-Vidal and Assenmacher, 1965) and quail (Tixier-Vidal *et al.*, 1967), and increases thyroidal uptake of ^{131}I (Tixier-Vidal and Assenmacher, 1965). At the peripheral level, castration of ducks increases the half-life of T4 (Jallageas and Assenmacher, 1972). Castration plus testosterone administration returned the t1/2 of T4 to normal, indicating that testosterone increases peripheral utilization of thyroidal hormones. In the quail, plasma T4 levels are depressed by testosterone treatment (Peczely *et al.*, 1979). In male birds that exhibit annual gonadal cycles, therefore, fluctuations in testosterone production may contribute to variations in thyroid function. Thyroidectomy of the red-vented bulbul (*Molpaster cafer*) or house sparrow (*Passer domesticus*) regardless of photostimulation results in a rapid collapse of the testes and, in the house sparrow loss of bill pigmentation (Lal and Thapliyal, 1982a, b). Thyroxine concentrations in the plasma measured prior to the onset of lay do not appear to be useful criteria for the selection of egg-laying strains (Sharp *et al.*, 1981).

1.8. Heat stress and the reduction of growth

Ambient temperature and humidity are important factors in the growth, food intake and food conversion rate. When we try to rear broiler chickens, or turkeys, or indeed any animals that are for meat at high ambient temperature, they grow slowly, and exhibit high mortalities (Al-Zujaly *et al.*, 1977). The effects of such environments on the performance of broilers have been examined by many scientists and quantified by a statistical analysis and assessment of the published literature. The results obtained from 15 papers which were reviewed by Howlider and Rose (1987), showed that an increase in environmental temperature decreases the live weight gains and food intakes

of broilers (Figures 10 and 11). Over the whole range of temperatures used in that review the relationship of these parameters with temperature was curvilinear, probably due to the broilers rapidly reducing food intake at the higher temperatures to reduce heat stress. Very often the reduction in performance is attributed to the birds rapidly reducing their food intake at higher temperatures to reduce heat stress (Cowan and Michie, 1977; Deaton *et al.*, 1978; Deaton *et al.*, 1984; Charles *et al.*, 1981; McNaughton and Reece, 1984; Howlider and Rose, 1987). However, when production is depressed at higher temperatures, the role of limiting food intake is not always clear, although pair feeding experiments (Fuller and Dale, 1979; Smith and Oliver, 1972) suggest that it rarely provides a complete explanation. Fuller and Dale (1979) found that the body weight gain (BWG) of heat stressed broilers was lower than of the pair-fed thermoneutral broilers examined between 4 to 7 weeks of age in three environments: a cool one, a hot one and a cool (the pair-fed) one in which birds were fed the same amount of food as the hot birds.

In the experiment conducted by Fuller and Dale (1979) to determine the influence of feed intake on performance of broiler-type chickens raised in a hot or cool environment, diurnal temperature cycles of 24 to 35 °C and 13 to 24 °C constituted the hot and cool environments, respectively. Chickens were fed the experimental diets *ad libitum* in both environments, and an additional group of chickens in the cold environment was limited to the amount of feed consumed by the chickens in the hot environment. The results indicate that growth was depressed by 25% in broilers maintained in the hot environment. However, when chicks maintained in the cool environment were fed the same amount of food as consumed by the chickens in the hot environment, their performance was reduced by only 16% compared to chickens fed on an *ad libitum* basis.

Moss and Balnave (1978) conducted a similar study using 18-day and 25-day old male chickens (unknown breed), maintained at temperatures of 22 and 30 °C for

Regression equations: x = Temperature (°C)

1. y (Food conversion ratio %) = $105.37+1.43(\pm0.721)x-0.15(\pm0.036)x^2+0.003\pm(\pm0.0006)x^3$
2. y (Body weight gain %) = $102.95-0.82(\pm0.092)x+0.09(\pm0.049)x^2-0.003\pm(\pm0.0007)x^3$

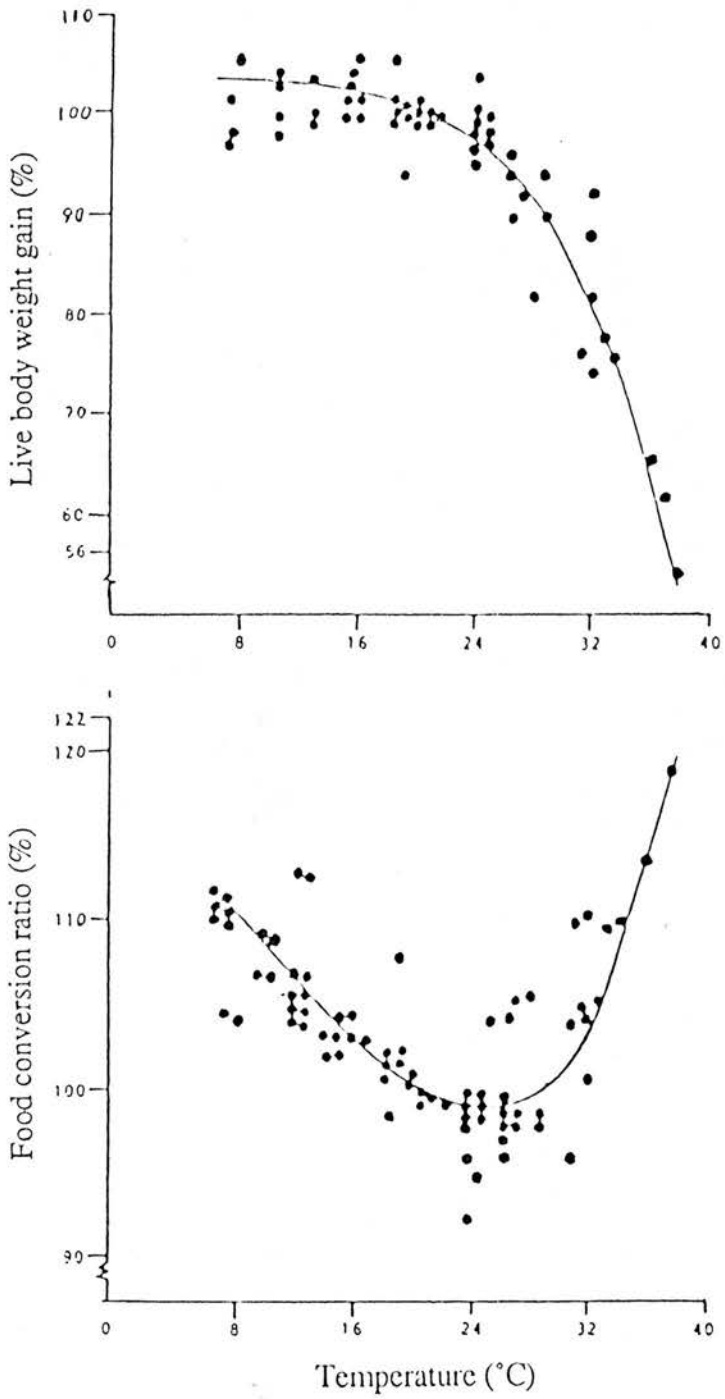


Figure 10. The effect of temperature on the body weight gain and food conversion ratio of as hatched broiler chickens. The values in the figures are observations from 15 reports in the literature expressed as a percentage of the performance obtained form the boilers kept at 21 °C in that experiment. Adapted from Howlider and Rose (1987).

Regression equations: x = Temperature (°C)

1. y (Food conversion ratio %) = $105.37+1.43(\pm0.721)x-0.15(\pm0.036)x^2+0.003\pm(\pm0.0006)x^3$
3. y (Food intake %) = $105.52+0.74(\pm0.227)x-0.05(\pm0.0005)x^2$

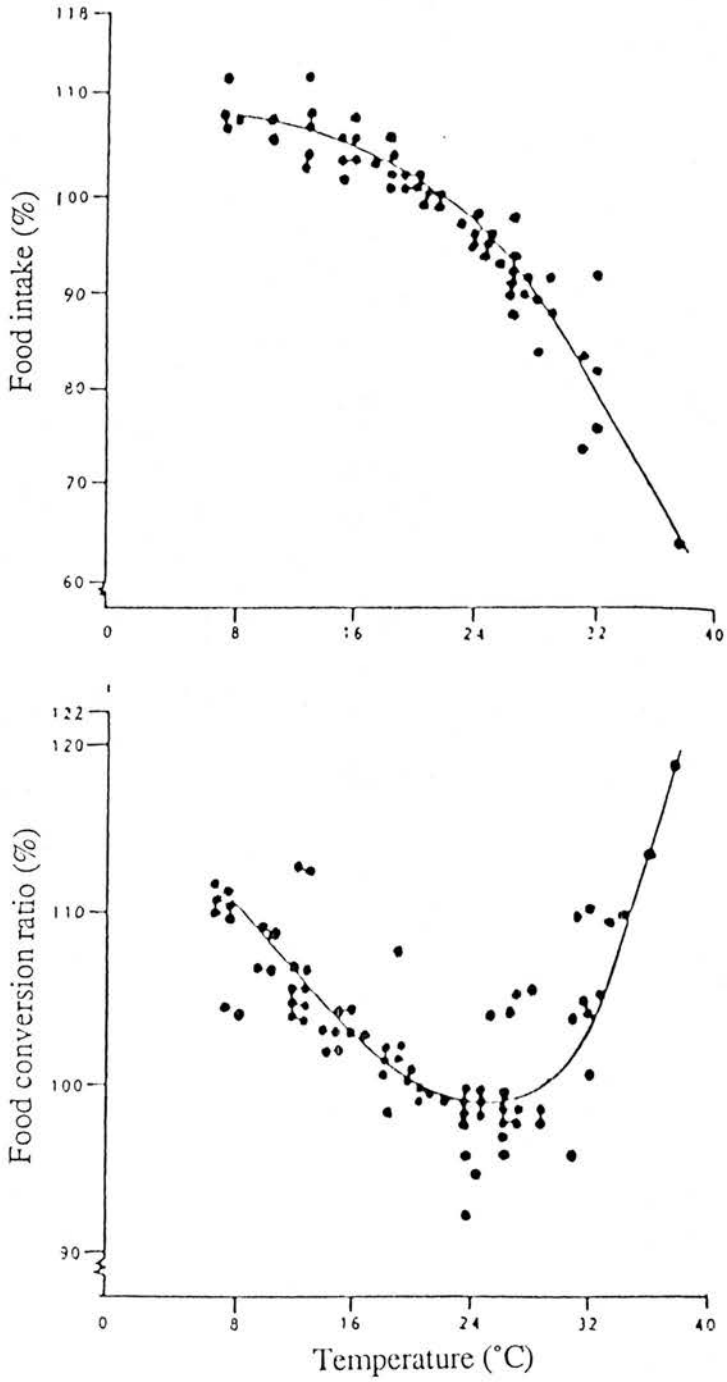


Figure 11. The effect of temperature on the food intake and food conversion ratio of as hatched broiler chickens. The values in the figures are observations from 15 reports in the literature expressed as a percentage of the performance obtained form the boilers kept at 21 °C in that experiment. Adapted from Howlider and Rose (1987).

16 and 28 days, respectively. Birds maintained in the two environmental temperatures were fed *ad libitum*, and some birds held at 22 °C were fed the same amount of food as consumed by chickens at 30 °C. The plasma thyroxine concentration and liver weight were measured during this period. As ambient temperature increased from 22 to 30 °C, liver weight of birds examined for 16 or 28 days, were deceased by 8 or 7%, and the plasma thyroxine concentrations were increased by 37 or 103%, respectively. When birds in the 22 °C environment were limited to the levels of food consumption of the 30 °C group, liver weights of birds examined for 16 or 28 days, were increased by 7 or 9%, respectively. The plasma thyroxine concentration was not increased but decreased by 12% when birds were pair-fed to the birds examined for 16 days in 30 °C, but was increased by only 50% when food was limited to the amount consumed by the birds examined for 28 days in 30 °C. This was associated with a 30% reduction in thyroid weight from 52 to 36 mg/kg body weight at the higher temperature, compared with birds kept at 22 °C and were limited to the levels of food consumption of the 30 °C group. The interpretation of the results was, however, difficult as they did not measure T3, and did not monitor ambient humidity, record body weight, or report which strain was used.

Clearly, the depressed growth of the heat stressed birds does not result from decreased food intake alone. The difference between the body weight gain of the heat stressed and pair-fed broilers might be caused by other factors rather than food intake. Smith and Oliver, (1972) conducted a study using laying hens maintained at temperatures of 21, 32 or 38 °C. Hens maintained in the three environmental temperatures were fed *ad libitum*, and some birds held at 21 °C were fed the same amount of food as consumed by chickens at 32 or 38 °C. Egg production was monitored during a 10-week experimental period. As ambient temperature increased from 21 to 32 and 38 °C, egg production was decreased by 7 and 52% and egg size was deceased by 2 and 18%, respectively. When birds in the 21 °C environment were limited to the levels of food consumption of the 32 or 38 °C groups, egg production

was reduced by 8 and 26%, respectively. Egg size was not decreased when hens were pair-fed to the hens in the 32 °C, but was decreased by 7% when food was limited to the amount consumed by the hens in the 38 °C environment.

The above results indicate that limiting food intake alone does not result in as severe a reduction in performance as maintaining the birds in a hot environment with the same level of food intake. Clearly, high environmental temperatures impose limitations on the performance of both broiler and laying hens that do not result from decreased food intake alone. These results raise the possibility that changes of thyroid function and reduced food consumption may account for some of the impairment in production.

The discovery of the ventromedial hypothalamic satiety centre and the lateral hypothalamic feeding centre led to the concept of food intake as a homeostatic function (Sykes, 1983). Response to food intake may according to Morrison (1959) be mediated by thermal gradient, the difference between the venous and arterial blood glucose level or by the concentration of circulating metabolites from endogenous fat stores in the bird.

Hurwitz *et al.* (1980) have shown that the efficiency of birds increases with increasing environmental temperatures to reach a maximum at 27°C. This is associated with the declining heat production with increasing temperature from 12 to 34 °C.

Food intake according to Freeman (1983) adapts to meet the energy requirement of the body and it is modified by ambient temperature with a response to changing requirements because metabolic rate varies inversely with temperature until the upper critical temperature is reached. Food intake is suppressed to avoid hyperthermia when chickens are exposed to continuous high environmental temperature (Smith and Oliver, 1971) and by reduced food intake birds undergo reduced metabolic heat production (MacLeod *et al.*, 1980b; Klandorf *et al.*, 1981).

High environmental temperature imposes limitations on the performance of the broiler chicken which does not depend only on food intake. The exposure of broilers to temperature stress is associated with alterations in protein metabolism as shown by the free amino acids pools in blood plasma (May *et al.*, 1972). Such alterations could represent either dietary effects or hormonal, physiological and other biochemical influences on the transport and metabolism of amino acids in tissues of the organisms affected by a stress related stimulus (Wannemacher, 1972). On the other hand, it has been demonstrated that stress related stimulus alters the pattern of protein synthesis which is governed by the flux and/or concentrations of free cellular and plasma amino acids (Wannemacher, 1972; Maruyama *et al.*, 1976).

Although high environmental temperatures do lower the birds food intake and other performance parameters, it does also affect the body's systems like the cardiovascular and respiratory systems, the nervous and endocrine system. Changes in the body's systems are mediated by both chemical and neural mechanisms. Respiration is one of the first parameters to change during hyperthermia and because the respiratory system is the prime source of temperature regulation during such conditions. It has been suggested that it may be this physiological mechanism that fails first when birds succumb to hyperthermia (Frankel *et al.*, 1962).

1.9. Strategies for reduction or alleviation of heat stress

Several methods have been tested to alleviate the effects of heat stress under such tropical conditions, among the most common are: the use of high nutrient density diets, carbonated drinking water, incorporation of fat into the diet, administration of vitamin C (Perek and Kendler, 1962, 1963) and more recently of vitamin E. The results observed so far are not conclusive and sometimes are contradictory.

Numerous reports in the literature show that management techniques concerning physiological mechanisms have been investigated taking into account

during heat stress. Three main methods of overcoming the detrimental effect of high temperatures are known. These are 1) control and improvement of the thermal environment with regard to physical and social factors; 2) selection of breeds with better heat tolerance, especially using breeds with tropically tested major genes; and 3) modification of diet. Where possibilities of improving management conditions are limited (Ota and McNally, 1963; Deaton, 1983) breeding concepts and dietary modifications gain in importance. The advantage of breeding concepts for reducing thermostress are described in detail by Horst and Petersen (1977), Horst (1981), Bordas and Mérat (1984), and Männer (1991, 1992).

Methods of reducing heat increment in heat stressed birds, including reduced energy intake at day time, reduced protein and amino acid, fat feeding, and other nutritional strategies, have been applied. Heat tolerance is labile and can be increased or decreased considerably by reducing or increasing energy intake. For example, Sykes and Fataftah (1980) demonstrated that the addition of maize oil to the diet of acclimatised laying hens led to a loss of heat tolerance because of the increment in energy intake.

1.9.1. Antioxidant capacities of vitamin C and E in stress protection

Heat stress may promote oxidative damage to cells through free radical formation. Excessive amounts of active oxygen results in lipid peroxidation and damage to organelles, cells and their membranes. In normal animals there is sufficient endogenous antioxidant capacity to remove active oxygen but this does not occur in stressed animals. Incorporation of vitamin E (α-tocopherol) in the diet may help to reduce lipid peroxidation because it interacts with selenium-containing glutathione-peroxidase to prevent the oxidative damage. Relationships with other compounds like ascorbic acid (AA, or vitamin C), have been proposed.

Heat stress may lead to a tissue damage due to changes in the acid-base balance, impaired immunological reactivity, changes in hormone secretion and possibly because of the over production of oxygen free radicals.

1.9.1.1. Heat stress and oxidative cell damage

It is well established that any form of stress can lead to a reduction in the animals' defence mechanisms or to a relative state of immunosuppression (Brown, 1967; McFarlane *et al.*, 1989).

It is also agreed that stress can lead to over production of oxygen free radicals OH^- and O^{2-} (Slater, 1984). In general high temperatures are required to break covalent bonds, but some bonds are relative unstable and break at temperatures of 30 to 50 °C. Free radicals may be generated by the cells in the immune system during stress or by certain metabolic pathways, e.g. eicosanoid synthesis.

Free radicals can cause metabolic disturbances and cell injury in a variety of ways, for example, if a reactive free radical is formed close to DNA then it may produce a change in the cell structure resulting in a mutation or cytotoxicity. They also cause profound changes in enzyme activity. Reactive free radicals may also damage cells by lipid peroxidation of polyunsaturated fatty acids (PUFAs) with direct effects on membrane structure. This is by far the most important damage produced by free radicals in the animal (Slater, 1984; Halliwell and Gutteridge, 1984). In heat stressed animals normal antioxidant capacity can be exhausted. Thus free radicals can initiate and propagate peroxidative damage to several cell constituents including PUFAs in cell membranes. Such decomposition can lead to the disruption of cell membranes and cause an increased leakage of enzyme such as creatine kinase (CK) and pyruvate kinase (PK) from tissue to plasma (Duthie *et al.*, 1989) This damage can be particularly serious in organs like the liver and muscle because of their high metabolic activity (Fowler, 1990).

1.9.1.2. The role of vitamin C in acid-base balance and in tissue membrane protection during heat stress

Several compounds have been examined that may alleviate the effects of high environmental temperatures on broiler performance. One previously tested compounds is ascorbic acid (AA) or vitamin C. Vitamin C was reported to improve the performance of poultry in a hot environment (Thornton and Moreng, 1959). There have been many studies on this subject in the past decade and the results are inconclusive (Scott, 1976; Moreng, 1980; Njoku, 1986; Njoku *et al.*, 1992; Whitehead *et al.*, 1992). There is no evidence that it is an essential nutrient for chickens maintained under thermoneutral conditions.

Ascorbic acid is accepted as a useful but nonessential ingredient of poultry diets since the 1920's, but the success of this substances for improving broiler performance during heat stress is variable. Supplemental AA has been reported to improve heat resistance and reduce mortality associated with elevated ambient temperatures in chickens (Thornton, 1962; Perek and Kendler, 1962, 1963; Ahmad *et al.*, 1967; Lyle and Moreng, 1968; Attia, 1976; Pardue *et al.*, 1985b). Broiler chickens can even select and adjust the proportion of AA supplemented and unsupplemented foods eaten to meet the requirements for AA according to environmental temperature (Kutlu and Forbes, 1993a), after they were taught to distinguish between different levels of AA in food by means of their colours. Addition of 1000 mg/kg AA reduced weight losses and mortality in birds up to 4 weeks of age following an heat exposure (Pardue and Thaxton, 1986; Pardue *et al.*, 1985b). However, no improvement in weight gain or survival was observed in other studies (Subaschandran and Balloun, 1967) when AA was provided at either 1000 mg/kg (Pardue *et al.*, 1985a; Stilborn *et al.*, 1988) or at 1% of the diet (Brown and Southern 1985). Thornton (1961); Scott (1975); Perek and Kendler (1963) speculated that AA synthesis in the chicken is reduced during periods of high environmental

temperatures. Al-Janabi *et al.* (1988). found that thyroid weight showed a significant increase in most 144-days-old layer chickens which ascorbic acid was given to and mixed with their ration at three concentrations: 30, 60 and 90 p.p.m. for 6 months. None of the studies described above reported plasma thyroid hormone levels of broiler chickens in which supplemental AA has been reported to improve heat resistance and reduce the mortality associated with elevated ambient temperatures. Because of the conflicting results on broiler growth performance observed in such studies, one of the objectives of the present experiment was to determine the effect of AA on plasma thyroid hormone levels and 5'-deiodinase activity in the broiler chickens exposed to heat stress.

1.9.1.3. The role of vitamin E in tissue membrane protection during heat stress

Vitamin E (α-tocopherol) is involved in several crucial metabolic processes. It mainly acts as an inter-and intra-cellular antioxidant protecting the unsaturated fatty acids both in the diet and in the cell membrane. It is also part of several metabolic pathways, and helps to maintain the integrity of blood vessels (Fowler, 1990). The intramembrane antioxidant properties of vitamin E may protect the sarcolema from lipid peroxidation due to free radical attack and minimise the associated loss of integrity and increased permeability (Masika, 1991).

The unsaturated double bonds of membrane polyunsaturated fatty acids (PUFAs) are inherently unstable and are readily attacked by peroxides and other forms of active oxygen. This process tends to produce a chain reaction and more free radicals and hydroperoxides are produced. Vitamin E acts as a scavenger of free radicals and prevents this explosive reaction (Putnam and Comben, 1987).

Vitamin E has also been associated with selenium (Se) in the prevention of lipid peroxidation. Se is an integral component of the enzyme glutathione peroxidase

(GSH-Px). This enzyme is a key component of the antioxidant system (Martensson *et al.*, 1991). Hoekstra (1975), has suggested that both vitamin E and Se have complementary roles in the prevention of oxidative damage. It is also important to recognise that, vitamin E and Se are not exchangeable. In the absence of adequate Se to form GSH-Px, cells will contain excessive peroxides which will attack unsaturated lipids in spite of the protection of adequate vitamin E. (Putnam and Comben, 1987).

A relationship between Vitamin E and Vitamin C has been proposed by McCay (1985). It was suggested that ascorbate either has a sparing action on tocopherols by itself acting as an antioxidant or acts on tocopheroxyl radical (the oxidised form of tocopherol) to remove the oxygen and so regenerate active tocopherol.

The inhibition of lipid peroxidation by antioxidants is well documented in the literature (Stuckey, 1962). The biochemical function of vitamin E (α-tocopherol) as an antioxidant rests on secure foundations (Tappel, 1953, 1968; Combs *et al.*, 1975).

There is considerable evidence that free radicals attack polyunsaturated fatty acids in cell membranes, and that the degradation of these fatty acids results in cell damage (Tappel, 1953). In addition, stimulation of phospholipase A2 by increased intracellular calcium may result in enhanced production of prostaglandins, leukotrienes but may lead to further free radical production. This process may further lead to membrane damage and thus enzyme leakage. It is considered that vitamin E would be one of the most effective compounds for protection against these cell impairments and principally this vitamin is associated with the protection of membranes (McCay *et al.*, 1972; Lucy, 1972).

In addition to its antioxidant properties, vitamin E is known to be an inhibitor of phospholipase A2 and may therefore further reduce the effects of a particular stressor upon muscle structure and function. In this way vitamin E affects arachidonic acid metabolism and prostaglandin function. Vitamin E affects prostaglandins by

means of a mechanism which is connected with polyunsaturated fatty acid metabolism and in particular arachidonic acid. Arachidonic acid is transformed by two enzymes lipoxygenase and cycloxygenase, into hydroperoxy fatty acids and endoperoxides respectively and prostacyclins, thromboxanes and prostaglandins are derived from the latter. The vitamin E acts on the lipoxygenase and therefore on the hydroperoxide production which modulates the cycloxygenase activity (Panganamals and Cornwell, 1982). However, it is not clear if the mechanism which modulates the prostaglandin biosynthesis is exclusively connected with the antioxidant property of vitamin E or if other mechanisms are involved (Panganamals and Cornwell, 1982).

1.9.2. Reduction of respiratory alkalosis by dietary modification

Evaporative heat loss becomes the major avenue of heat loss as ambient temperatures (T_a) approaches and exceeds body temperatures (T_b). Control over the rate of evaporation is modulated through the rate (frequency) or the amplitude (tidal volume) of respiratory EWL. On the other hand, cutaneous water loss is a passive mechanism, where little control can be exerted except possibly by behaviour or by postural changes to reduce the boundary layer over selected skin surfaces or by increasing blood flow to the skin to raise the skin temperatures (T_{skin}), thereby widening the vapour pressure gradient. Although respiratory EWL is an effective mechanism for heat dissipation, it renders birds vulnerable to blood acid-base imbalance because the increased ventilation of the lungs reduces the pressure of alveolar CO_2, resulting in hypocapnia and blood alkalosis. Therefore, during heat exposure a conflict arises between thermoregulatory control, which increases ventilation to enhance respiratory EHL, and chemical control, which minimises ventilation of the lungs to maintain blood acid-base homeostasis.

When exposed to sufficiently high heat stress to induce panting, birds are vulnerable to respiratory alkalosis (Marder and Arad, 1989). Increasing respiration

rate lowers the partial pressure of carbon dioxide (pCO_2) in the lungs and circulation, which in turn raises blood pH. In chronically heat stressed birds, the concentration of blood HCO_3^- may also decrease in order to reduce blood pH back to the normal level. When chickens were exposed to 35 °C ambient, respiration rate increased from about 36 to about 150 breaths per minute, both blood pCO_2 and HCO_3^- decreased, and blood pH was elevated from about 7.53 to about 7.57 (El-Hadi and Sykes, 1982). Exposure to 41 °C ambient elevated respiration rate to about 180 breaths per minute and blood pH rose from 7.50 to 7.65. At this stage in panting it is useful to separate the action of thermal and chemical receptors and their influence over respiration rate and tidal volume. To this end, Barnas *et al.* (1981) artificially adjusted arterial pCO_2 levels in chickens by a unidirectional ventilation technique with set levels of pCO_2 in the inspired air, while simultaneously observing respiratory movements as T_b rose during heat exposure. At a given T_b, artificially induced blood alkalosis increased respiration rate and reduced tidal volume. On the other hand, when alkalosis was averted by artificially ventilating with normal pCO_2 under intense hyperthermia, respiration rate was depressed and tidal volume was increased compared to the respiration rate and tidal volume under intense hyperthermia in the normal chicken.

One of the first signs of heat stress in birds is an increase in the respiratory rate i.e. thermal polypnea or panting which is related to the increment in body temperature. Panting is the main mechanism of latent heat dissipation during heat stress. In addition to the beneficial effects of panting, it may also have detrimental effects on the bird. Thermal panting can induce a complex train of events in which pulmonary gas exchange, blood gas transport, tissue gas exchange, cellular metabolism and acid-base balance may each be affected (Kazemi and Johnson, 1986).

Data given by Teeter *et al.* (1985) indicate that blood alkalosis limits growth rate of broiler chicks reared under chronic thermal stress and weight gain depressions attributed to thermal stress can be partially alleviated dietarily. Numerous methods of

adding different materials to diets in order to decreased blood pH to normal during heat stress are reported in the literature and show that most of the techniques can increase body weight gains. For example, adding 0.3% or 1% ammonium chloride (NH_4Cl) to diets decreased blood pH to 7.194 and increased body weight gains by 9.5 and 25% respectively. Supplementing drinking water with 0.2% NH_4Cl reduced panting phase blood pH to normal values and increased live weight gain (23%) and feed efficiency (7.7%), or, supplementing drinking water with 0.15% KCl also increased live weight gain (46%) and feed efficiency (15.4%) but did not affect blood pH when chickens reared under continuous heat stress (35 °C, 70% relative humidity) (Teeter *et al.*, 1985; Bottje and Harrison, 1985a; Teeter and Smith, 1986; Bottje and Harrison, 1986; Smith and Teeter, 1987; Bottje *et al.*, 1989; Donoghue *et al.*, 1990b; Whiting *et al.*, 1990; Whiting *et al.*, 1991a,b).

1.9.3. The dietary proteins in heat stress protection

Food intake in a warm environment may be enhanced by supplying adequate, but not excessive, levels of protein having a good balance of amino acids. The exposure of broilers to temperature stress is associated with alterations in protein metabolism as shown by the free amino acids pools in blood plasma (May *et al.*, 1972). Such alterations could represent either dietary effects or hormonal, physiological and other biochemical influences on the transport and metabolism of amino acids in tissues of the organisms affected by a stress related stimulus (Wannemacher, 1972). On the other hand, it has been demonstrated that a stress related stimulus alters the pattern of protein synthesis which is governed by the flux and/or concentrations of free cellular and plasma amino acids (Wannemacher, 1972; Maruyama *et al.*, 1976). Protein turnover in heat stressed birds was higher than in thermoneutral environments, a high protein diet for heat stressed birds was , therefore, suggested. This suggestion was, however, questioned by the fact that heat tolerance can be increased considerably by reducing protein and amino acid content of the diet in

order to reduce heat increment in heat stressed birds (Sykes and Fataftah, 1980) because heat production derived from the breakdown of proteins was higher than of carbohydrates and fat.

1.9.4. The dietary fatty acids in heat stressed birds

Food intake in a warm environment may be enhanced by supplying adequate, but not excessive, levels of fatty acids. Increasing dietary energy concentration by the substitution of fat or oil for carbohydrate may stimulate energy intake (Austic, 1985).

High temperature is a negative stimulus for food intake (Houpt *et al.*, 1979). It may be expected, therefore, that diets which have a high heat increment will be detrimental in high environmental temperatures. Protein contributes more to the heat increment than do carbohydrates or fat (Scheibel *et al.*, 1979). Furthermore, the use of diets imbalanced with regard to amino acids results in increased protein catabolism and increased heat production (March and Biely, 1972). Therefore, minimising protein levels and improving the balance of amino acids should minimise the heat increment and thereby reduce the amount of heat which must be dissipated.

Another approach to improving food consumption and high environmental temperatures has met with success. Increasing the energy density of the diet by the addition of fat may stimulate egg production or growth (Fuller and Dale, 1979; Dale and Fuller, 1979; Reid, 1979). The mechanism by which fat stimulates performance under these conditions is not understood. There were no significant advantages of a high-fat diet in terms of enhanced heat tolerance or a high-fat diet fed at high ambient temperatures confers no food intake advantage beyond that found in cooler conditions (Persons *et al.*, 1967; Kubena *et al.*, 1972, 1973; MacLeod, 1990, 1992). MacLeod (1992) found that energy intake is significantly increased in broilers at 32 °C by the addition of fat. The effects of diet composition were observed when the same birds were taken from 20 to 32 °C. The tendency for energy intake to increase with dietary

energy concentration was less at 32 than at 20 °C. The lower heat increment associated with high-fat diets conferred relatively less of an advantage of higher intake at 32 than 20 °C. Although there was a wide range of energy intakes as multiples of maintenance energy requirement, there was no indication that variation in heat production (HP) was used as a mechanism for control of energy retention; as 20 °C, variations in energy retention and body composition remained the main responses to variations in dietary crude protein (N x 6.25; CP) concentration and crude protein (CP - g/kg) : metabolisable energy (ME - MJ/kg) ratio (MacLeod, 1990, 1992). It is possible that the higher fat content of the diet contributes to a reduced heat production, as fat has a lower heat increment than either protein or carbohydrates. Although the HP of fed birds was significantly affected by dietary energy source, there was no evidence for regulatory diet-induced thermogenesis as energy intake increased (MacLeod, 1990, 1992). When fat is added to the diet, it also appears to increase the energy value of the other feed constituents (Scheibel *et al.*, 1979; Mateos and Sell, 1981). Fat decreases the rate of food passage in the gastrointestinal tract, perhaps increasing the efficiency of energy digestion (Mateos *et al.*, 1982).

Apart from trying to stimulate energy intake by increasing dietary energy concentration, the substitution of plant oil for carbohydrate, may also provide adequate levels of essential fatty acids. The requirement for essential fatty acids is satisfied by 1.2% linoleic acid in chick starter diets, 0.8% in grower diets, and by 1.2% in diets for laying and breeding hens. The dietary concentration of this nutrient should also be adjusted to compensate for changes in food intake at high environmental temperatures (Austic, 1985).

1.9.5. Feeding thyroid hormones

When young broilers are fed T3 or T4 (0.5 or 0.6 ppm) and then heat stressed, their survival time is significantly shortened as compared to controls'. The shortening of survival time is much greater for T3 than T4, and feeding T3 caused a reduction in

serum T4 concentration, but dietary T4 had no effect on serum T3 concentration (May, 1982).

1.9.6. The role of reduced heat production in heat tolerance

A few papers reported some management techniques could reduce the effect of heat stress. Some of these approaches have included acclimation of the birds to heat (Hutchinson and Sykes, 1953; Reece *et al.*, 1972; Bohren *et al.*, 1982a,b; Teeter *et al.*, 1992), fasting (McCormick *et al.*, 1979; Teeter *et al.*, 1987), preconditioning by handling (Bowen *et al.*, 1984), and exposing birds to mild heat stress for 24 h at 5 days of age (Arjona *et al.*, 1988). As we are aware, birds under such management techniques could reduce their heat production.

Although the mechanisms are not yet understood, it is obvious that the potential of zinc bacitracin (ZBA) in reducing heat production of fed hens is important for birds exposed to heat stress. Männer and Wang (1991) suggested that the lower body temperature under heat-stress conditions can be used for describing the stress diminishing effect of ZBA. Overall, addition of ZBA improved heat tolerance time by 23.7% for chickens kept under normal temperature conditions and by 51.2% for chickens acclimatized to 34 °C. ZBA-treated hens had a significantly higher food intake under extremely high temperatures compared with untreated hens. 100 mg ZBA/kg feed decreased the adverse effect of exposing hens to a constant high temperature (34 °C) (Männer, 1991).

It is also of interest that 26- to 29-day-old chicks reared at T_a = 22 to 24 °C and fasted for 24 hr prior to exposure to T_a = 41 °C were able to maintain their T_b for a shorter time and showed earlier heat prostration when they had received a diet with 0.35% available phosphorus than chicks which had received 0.55% available phosphorus, with heat prostration occurring at the same concentration of inorganic

phosphorus in the plasma (McCormick *et al.*, 1980a). Similar results were obtained for survival rates of such chicks fasted for either 24 or 48 hr prior to exposure to the hot environment (McCormick and Garlich, 1982). One of the consequences of feeding the low-phosphorus diet was a lower plasma Ca^{2+} concentration (7.8 mg/100 ml) in the blood 90 min after heat exposure than in chickens fed the adequate diet (9.7 mg/100 ml) (McCormick and Garlich, 1982). It is curious that in a factorial experiment with 0.35 and 0.55% phosphorus and 0.3 and 1.0% calcium as the variables, the highest survival times of 4-week-old chicks fasted for 24 hr and exposed to high T_a were 156 min for the adequate phosphorus, adequate calcium; 250 min for the low phosphorus, low calcium; and 266 min for the adequate phosphorus, low calcium diet (McCormick *et al.*, 1980b). In these experiments the concentration of Ca^{2+} in plasma of chicks prior to fasting was negatively correlated with survival time ($r = 0.48$, $p<0.001$), whereas the inorganic phosphate (Pi) concentration in the plasma was positively correlated with survival time ($r = 0.51$; $p<0.001$) with the Ca^{2+}/Pi ratio showing a negative correlation with survival time ($r = -0.41$, $p<0.001$) (McCormick *et al.*, 1980b).

Experiments by Edens (1976) showed that intravenous injection of 2 ml of a 5% Ca^{2+} solution did not affect the T_b response to exposure to $T_a = 45$ °C of 3-week-old chickens although the T_b at $T_a = 24$ °C was significantly lower in such Ca^{2+} -injected chickens than in controls kept at $T_a = 24$ °C. Injection of a 5% Na^+ solution was followed by a significantly greater increase in T_b during exposure to $T_a = 45$ °C than in controls at the same T_a, whereas at $T_a = 24$ °C no significant difference in T_b was found in comparison with the controls. The relationship between the concentrations of Ca^{2+}, Na^+, and Pi in plasma and T_b regulation and survival time needs further study. The multitude of effects of corticosterone on important physiological parameters which may have a function in survival during heat exposure make it a matter of speculation as which particular effects have a crucial function. It is

attractive to speculate that one of the crucial functions is the maintenance of Pi and of Ca^{2+} concentrations in the blood, thus protecting against cardiovascular failure.

There have been many observations concerning the effect of the heat stress on broiler chickens and possible methods of reducing the effect of heat stress in order to improve broiler productivity. The interrelationship between the broiler productivity and the underlying physiological regulation of the growth process has also been studied. However, little precise information is available as to the extent to which hormonal changes are related to decreased growth rate in broiler chickens exposed to high environmental temperatures. Similarly little is known of the relationship between methods used to improve broiler production during hot weather and the hormonal profile of broiler chickens. To date very few reports have reported how hormonal secretion affects the growth rate in broiler chickens exposed to high environmental temperatures.

The major aim of this project was to characterise the thyroid and growth hormone responses in young broiler chickens chronically exposed to different quantified thermal loads. In these studies, attention was focused upon changes in the plasma concentrations of thyroxine, triiodothyronine and growth hormone as well as the hepatic 5'-monodeiodinase activities *in vivo* in broiler chickens during chronic heat stress. Circulating hormone concentrations have been examined and the control of peripheral T3 production has been addressed. The possible roles of endocrine adaptations in heat stress induced changes in growth have been considered. The effects of nutritional and genetic strategies for the improvement of growth rate during heat stress upon thyroid hormone and growth hormone responses have also been investigated.

Chapter Two

Materials and methods

2.1. Animal housing and husbandry

Included in this section are general descriptions of methods and procedures common to all experiments. Specific details of individual experiments and modifications of general techniques are provided in the appropriate sections. Birds of all strains under investigation were initially reared and maintained in the Institute's facilities. All birds were reared under standard husbandry procedures in cages at the specified ambient temperatures. The birds received a standard commercial broiler diet and tap water.

2.1.1. Birds

Commercial female broiler chicks (1 day old) were purchased from Ross Poultry (G.B.) Ltd., Inverurie, Aberdeenshire, UK. and used up to 6 weeks of age. They were maintained in brooders (29±3 °C) for 20 days and vaccinated against Gumboro disease at 7 days old. Each bird was identified by a numbered wing band. A 14L: 10D photoperiod was employed in the brooders, with an experimental lighting pattern of a 23L: 1D photoperiod beyond 20 days of age.

The stock of naked neck broilers, which were chickens homozygous for the naked neck gene (Na/Na) (Figure 12), was originally obtained from Holland in the early 1980's. The line was maintained by inter-crossing for several generations in the Institute. Their body weights were, therefore, lower than modern broiler chickens (Figure 13).

The naked neck gene (Na), is a genetic mutation resulting in approximately 30-40% reduced feather covering (Mérat, 1986). Na is an incompletely dominant gene.

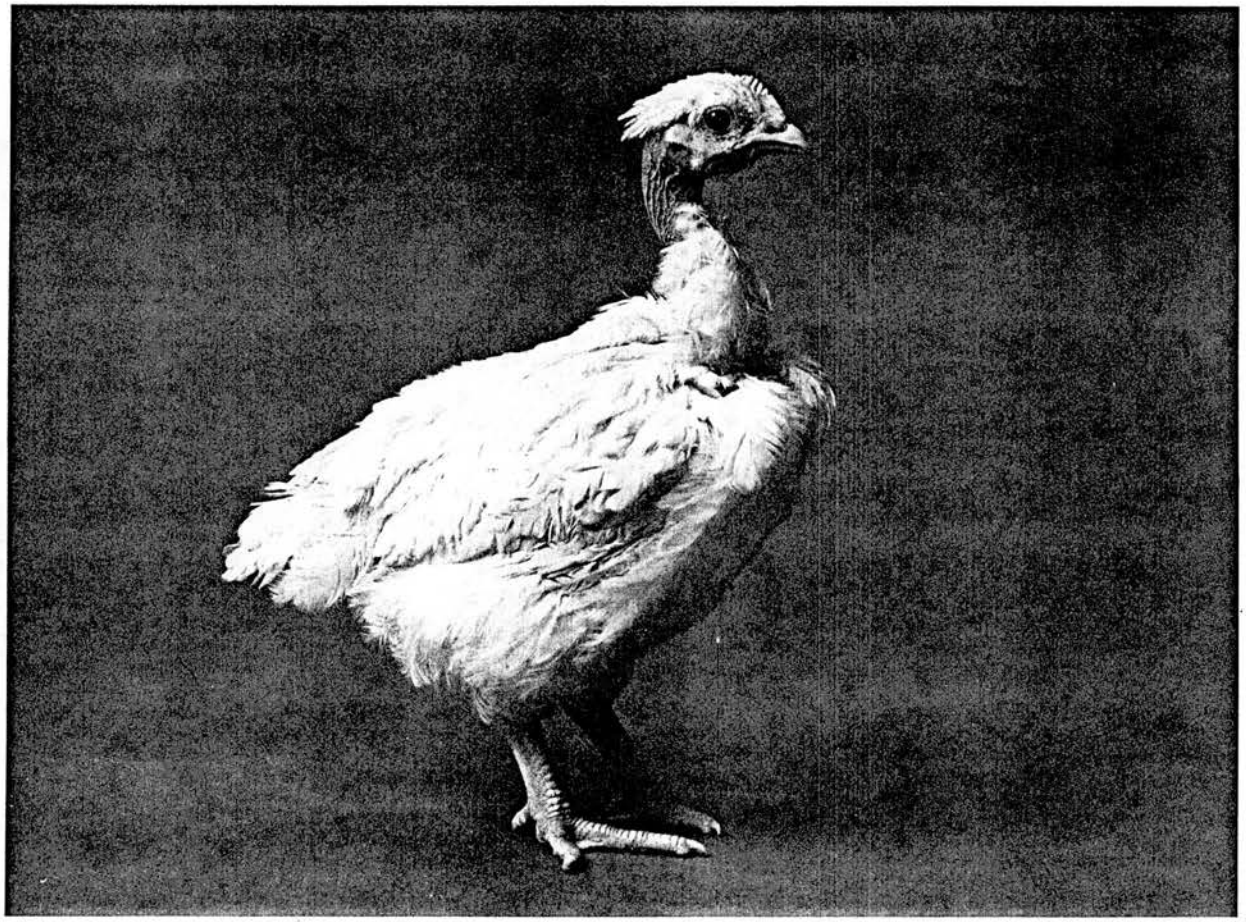


Figure 12. **Forty one days old female broiler chicken expressing the naked neck gene (Na/Na).**

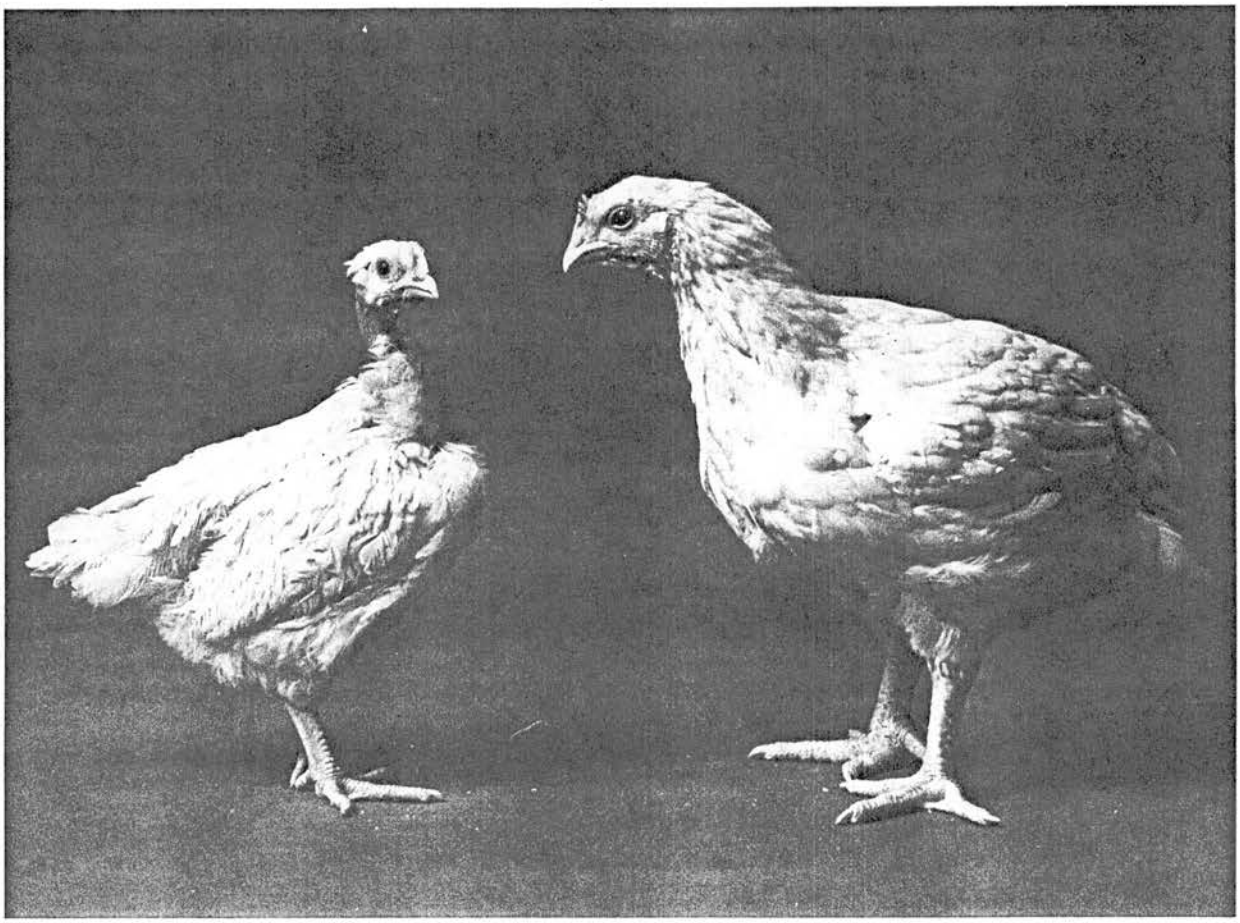


Figure 13. Comparison of body size between commercial and naked neck female broiler chickens at age of forty one days.

Heterozygous Na/na chickens, therefore, have less feather cover than homozygous normal (na/na) birds, but more feather cover than homozygous Na/Na birds. The genetic approach to improving performance of heat stressed birds may benefit from the use of naked neck gene by altering the extent of feathering. It has been predicted that Na chickens will produce a 9%-12% improvement in growth rate, a 6%-9% better egg weight and will increase food consumption in high environmental temperatures (Mérat, 1980; Mérat *et al.*, 1974; Mérat, 1986; Cahaner *et al.*, 1992, 1993), but may prove disadvantageous at normal temperatures (El-Atter and Mérat, 1985). The Na chicken has been found to have flexibility in thermoregulation and a consistently greater survival rate under heat stress.

The Na chickens were reared and maintained under the same conditions described for normal birds.

At 20 days of age, birds of all strains were transferred to three controlled climate chambers and caged individually, and exposed to the specified climatic conditions through control of temperatures and relative humidities.

2.1.2. Controlled climate chambers

Three controlled climate chambers (3.5 x 2.0 x 2.0 m) were used. The chambers were maintained at different climatic conditions. Temperature could be controlled to ±0.2 °C within the range -5 to +40 °C and a relative humidity (RH) to ±5 % between 10 and 100 %.

The chamber temperature and relative humidity were measured three times every day at 09:00, 13:30 and 18:00, using the dry and wet bulb thermometer, the whirling hygrometer (Casella London, England) was used to determine the relative humidity. The ambient temperature and relative humidity were also measured continuously using a Vaisala probe and recorded every 10 minutes by Data Logger (1200 Series Squirrel Meter/Logger, Grant Ltd., UK) for three days during each

experimental period. The data recorded by Logger can be transferred to computer by 20/20™ programme (Access Technology Ltd, Buckinghamshire, UK).

2.1.3. Body temperature, feed intake and growth monitoring

Food intakes from each individual bird were recorded every morning between 09:00-10:00.

Birds were fed every day and weighed every 2nd day at approximately 10:00-11:00 am for the whole period of 21 days. Records of feed intakes and bird weights were obtained. The mean values of body weight and daily weight gains for the experimental groups of birds were calculated by Minitab. The feed conversion efficiency and growth rate were plotted by KaleidaGraph (KaleidaGraph™ version 2.02, Oct. 1989, ©Abelbeck Software).

Birds were initially accepted or rejected by observation of daily body weight gain and food intake. Those birds which were physically damaged (clipped or crushed by cages) during experiments were rejected on this basis.

The birds' body temperatures were measured every two days using an electronic rectal probe inserted 5 cm into the rectum.

2.2 Collection of blood samples

Blood samples were obtained by venipuncture of the brachial vein. 2.5 ml samples were taken into heparinised syringes (rinsed with heparin in physiological saline 50 units/ml, Pularin Evans Medical) and immediately transferred to heparinised tubes (3 ml Teklab). Bloods were placed on a roller (Spiramix 5 roller, Jencons (Scientific) Ltd., Cherrycourt Way Ind. Estate, Leighton Buzzard, Beds) until they were centrifuged at 1500 g for 10 minutes (Denley BS 200 centrifuge) to obtain plasma. This technique enabled up to 20 serial samples to be taken in a 40 minute period. Blood was always withdrawn within 2 minutes of removing a bird from its

cage. Plasma was stored at -20 °C prior to assay for GH, T4 and T3.

2.3. The hormonal assay

2.3.1. The T4 and T3 radio-immuno-assay

2.3.1.1. Principle of the T4 or T3 radio-immuno-assay method

A constant volume of T4 or T3 antiserum is reacted with varying amounts of unlabelled hormone (standard solutions or unknown samples) and with a constant amount of radioactive tracer.

Reagent solutions are directly pipetted into chromatographic columns, capped at bottom and containing an immunoadsorbent (Sepharose gel to which a second antibody is covalently linked).

The reaction mixture is kept separated from the gel bed by a porous polythene disc. Therefore, in this procedure step, the small column acts as a test-tube where a classical liquid-phase radioimmunoassay reaction takes place. The separation of the antibody-bound antigen is performed by removing bottom caps to allow the reaction mixture to pass through the solid-phase matrix: antigen-antibody complex is bound to the immunoadsorbent by affinity chromatography while the unbound hormone is eluted by washing the column with buffer and discarded. The column radioactivity is then counted, the standard curve is constructed and the T4 or T3 unknown concentration in samples is read by interpolating from the standard curve.

2.3.1.2. The Total T3 and T4 radio-immuno-assay procedure

Plasma levels of total T3 and T4 were measured using commercially available kits (Liso-phase column affinity chromatography for bound/free separation assay). These commercially available kits were made by SCLAVO S.p.a. (RIA Department, SCLAVO S.p.a., Techno Genetics, Italy) and obtained from Metachem Diagnostics

Ltd, UK. The kits were stored at 4 °C until required for assay. The radio-immuno-assay procedure was a modification of the standard method using the above commercially available kits as described by Mitchell and Raza (1986b). Radio-immuno-assay tubes were counted on an LKB Wallac 1277 Gammamaster Automatic Gamma Counter (WALLAC Oy 20101 Turcu 10, Finland) for a time sufficient to accumulate > 70,000 counts in the total count tube. The sensitivities of the assays, defined as the concentration resulting in a response from the minimal detectable doses of T4 and T3 which were significantly different from zero ($p<0.05$) were 0.2 and 0.1 ng/ml, respectively. The T4 assay had an intra-assay coefficient of variation of 1.4 % while the corresponding value for the T3 assay was 2.5%. The inter-assay coefficients of variation were 2.0 % (n=10) for T4 and 5.3 % for T3. The non specific binding (NSB) was < 2%. The cross-reactivity of anti-T3 serum with the L-thyroxine analyses was < 0.05%. The cross-reactivity of anti-T4 serum with the L-Triiodothyronine analyses was < 0.16%. The cross-reactivity of both anti-T3 serum and anti-T4 serum with the 2.5-Diiodo-Dl-thyronine analyses was < 0.25%.

2.3.2. The growth hormone (GH) assay

Plasma levels of GH were measured by the method of Goddard *et al.* (1988) using a homologous double antibody radioimmunoassay (RIA). The principle of the GH radio-immuno-assay similar with T4 and T3 radio-immuno-assay as described in Section 2.3.1 (page 115), except anti-cGH serum was raised in a rabbit, which produced antiserum against cDNA -derived cGH, whilst anti-T4 and anti-T3 serum were raised in sheep.

For comparison some plasma concentrations of growth hormone were also measured by the method of Houston *et al.* (1991) using an enzyme-linked immunosorbent assay (ELISA) based on two monoclonal antibodies against cGH (Goddard *et al.*, 1987), because the GH radioimmunoassay has the disadvantages of using radioisotopes and a 3-day period before results are available. The ELISA was

highly specific for cGH and showed no cross-reactivity with other pituitary hormones (Houston *et al.*, 1991). The ELISA reproducibly measured cGH concentration. The principle of the ELISA is similar to that of the RIA, except the enzyme-linked detection system, which measured the optical density (read at 490 nm in a MR700 plate reader) from reaction with streptavidin peroxidase cojugate, was employed rather than radioactive detection system.

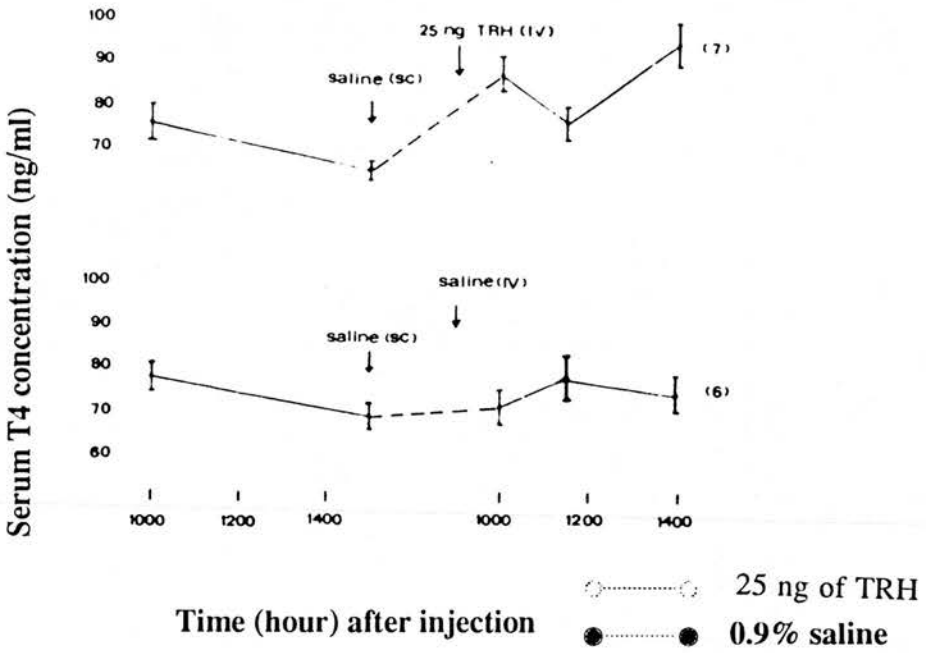
2.4. The function of the hypothalamo-pituitary-thyroid-liver axis

2.4.1. Injections of thyrotrophin releasing hormone (TRH)

After 21 days period (at 41 days of age) of exposure to the specified climatic conditions through control temperatures and relative humidities, some of the birds from each treatment were used for testing the function of the pituitary- thyroid axis and of stimulation of GH secretion and peripheral conversion of T4 to T3 (Mitchell, 1987a, 1988). Broilers were injected subcutaneously with thyrotrophin releasing hormone (Sigma Chemical Co., UK) in physiological (0.9%) saline on the basis of 10 μg/kg body weight (Mitchell and Raza, 1986b) in the leg near the femoral vein. Figures 14 and 15 showed that chickens' T4, T3 and GH levels were not affected by injection with physiological (0.9%) saline alone (Kühn and Nouwen, 1978; Harvey *et al.*, 1978).

About 2.5 ml of blood was collected directly from the wing vein into heparinised syringes just before injection and at 40 minute intervals for 160 minutes thereafter. Plasma was separated by centrifugation and stored at -20 °C until required for assay.

The responses of T4 concentration after TRH injection indicate the function of the hypothalamo-pituitary-thyroid axis in releasing T4, possibly reflecting the function of the pituitary in releasing thyrotrophin (TSH). The responses of plasma T3 concentration after TRH injection indicate the function of the hepatic 5'-



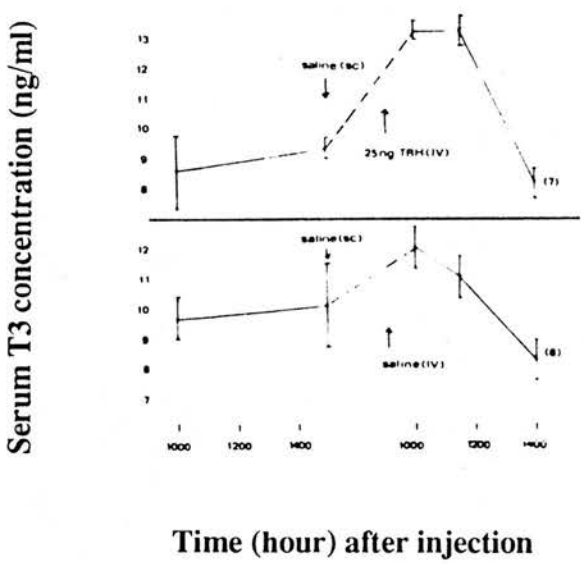


Figure 14. The influence of 25 ng of TRH on serum T4 and T3 concentrations in saline pre-treated 40-day-old cockerels (mean ± SEM). The number of animals is given in parentheses. Adapted from Kühn and Nouwen (1978).

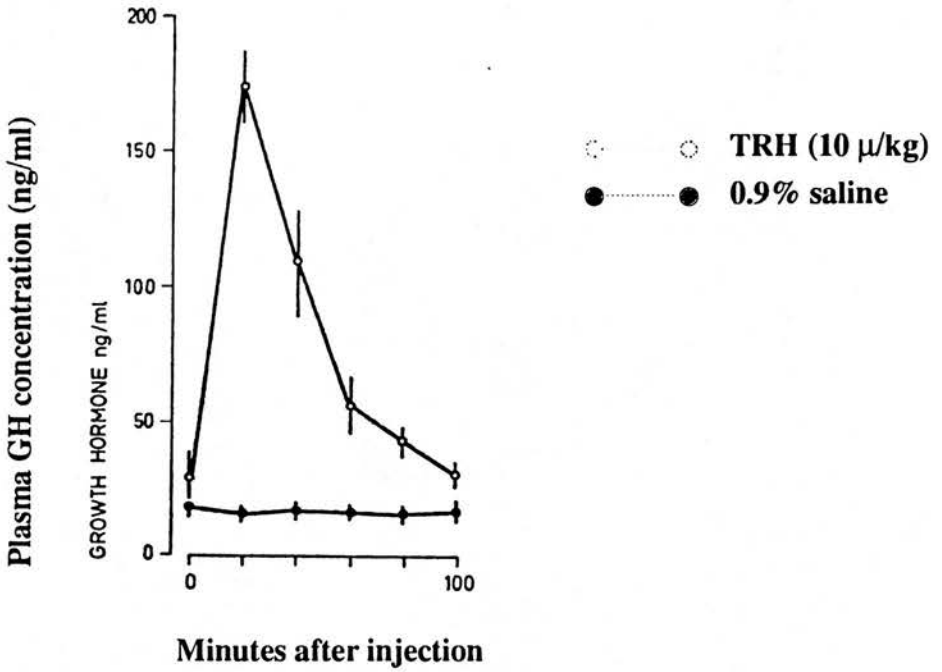


Figure 15. The influence of injections of TRH (10 μg/kg) in saline and 0.9% saline alone on plasma GH concentrations in three-week-old cockerels (mean ± SEM, n=5). Adapted from Harvey *et al.* (1978).

monodeiodination *in vivo* in the conversion of T4 to T3, possibly reflecting the function of the pituitary in releasing GH predicted by its functions in regulation of the hepatic 5'-monodeiodination *in vivo*. The responses of GH concentration after TRH injection indicate the function of the hypothalamo-pituitary axis in releasing GH *in vivo*, possibly reflecting the half life of GH.

Due to the complex feedback system of hypothalamo-pituitary-thyroid axis in control of thyroid functions (see Section 1.3, Page 15 for details), the responses of T4, T3 and GH concentration after TRH injection may also reflect the sensitivity of the hypothalamo-pituitary axis responses to positive and negative feedback of these hormones in releasing TSH and GH *in vivo*.

2.4.2. Injections of cDNA -derived chicken growth hormone (cGH)

After 21 days period of exposure to the specified climatic conditions, some birds from each treatment were used for the function of the pituitary-thyroid-liver axes test. Broilers were injected subcutaneously with cDNA -derived chicken growth hormone (cGH, obtained from Goddard) in physiological saline on the basis of 15 μg/kg body weight in the leg near the femoral vein.

About 2.5 ml heparinised blood samples were obtained at 11:00 am from all birds by venipuncture (brachial vein) before they received a subcutaneous injection of cGH to test the function of the GH in stimulation of secretion peripheral conversion of T4 to T3. Then, four further samples were taken at 40 minutes intervals post-injection. The blood plasma was prepared by centrifugation at 1500 g for 10 minutes and stored at -20 °C prior to assay for chicken GH, T4 and T3.

The concurrent responses of plasma T4 and T3 concentration after GH injection reflect the function of GH in regulation of the hepatic 5'-monodeiodination and control of thyroidal secretion *in vivo*. The responses in plasma GH concentration after GH injection may also be regarded as reflecting the rate of elimination (half life)

of GH.

2.5. Statistical analyses

2.5.1. Hormone data analyses

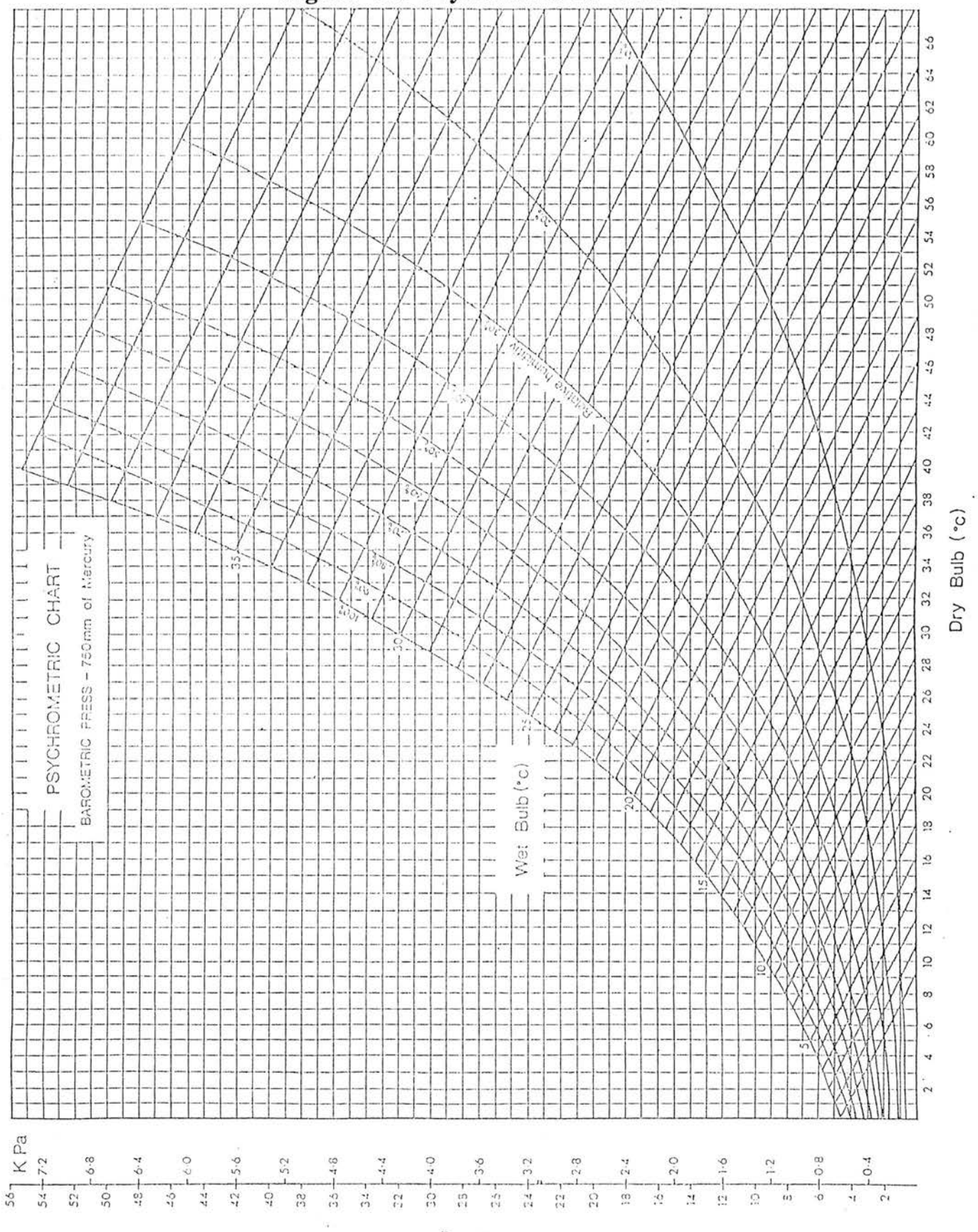
For all the hormone assays described in this thesis, estimates and their 95% confidence limits were calculated using a computer program "AssayZap" (Universal Assay Calculator) developed by P.L.Taylor (1987-91), published by BIOSOFT, Cambridge UK, and was modified as a radioimmunoassay data processing program package by the Institute. The computer program was designed to linearise the standard curve using a logit-log transformation. The calibration line was used as the standard curve for determining estimates and 95% confidence limits for the control and unknown samples. The program also provided an estimate of the minimal detectable dose (measure of the sensitivity of the assay and defined as the smallest hormone concentration for which the response is different from the "0" standard). The relative simplicity of the radioimmunoassay procedure enabled all the samples of a particular study to be assayed together.

2.5.2. Environmental data analyses

The water vapour densities and apparent equivalent temperatures during each experimental period were calculated according to the mean values of the ambient temperature and relative humidity by the following in house Basic programme:

```
100 OPEN "O",#1, "EQUIV_T"
120 SCREEN 0
130 COLOR 1,3
135 PRINT"T", "F","RH", "VD", "L"
140 FOR T= 0 TO 40 STEP 1
160 FOR RH= 0 TO 100 STEP 5
170 F=(((9/5)*T)+32)
210 D=(T+273.16)
220 REM LOG CONVERSION
230 Z=(LOG(D)/LOG(10))
240 Y=(30.5905-(8.2*Z)+(.0024804*D)-(3142.31/D))
250 P=10^Y
300 Q=P*(RH/100)
```

Figure 16. Psychrometric Chart



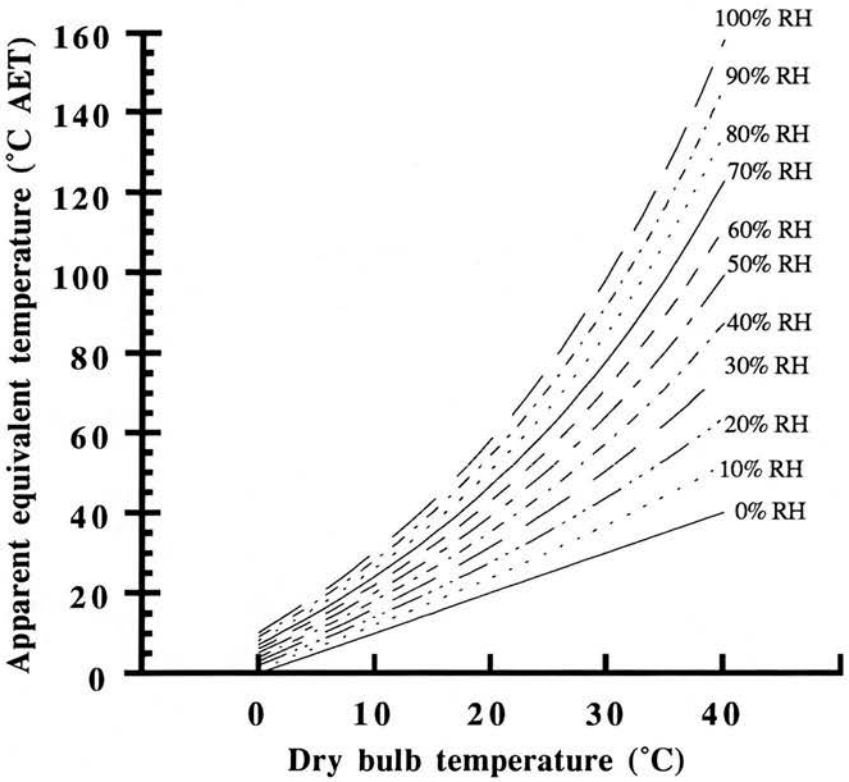


Figure 17. Apparent Equivalent Temperature (AET)

This psychrometric chart gives the complex relations between dry bulb temperature (°C), relative humidity (%) and apparent equivalent temperature (°C).

```
310 ET=((1006*T)+(.62194*(Q/(101.325-Q)))*(2500580!+(1875.69*T)))/1000
330 VD=(2170*Q)/(D)
350 E=Q*10
360 J=(6.363601E-04*D)+.472
370 K=(J*.93)
380 L=T+(E/K)
400 PRINT T,F,RH,VD,L
410 PRINT#1,T,F,RH,VD,L
420 NEXT RH
440 NEXT T
```

The above Basic programme is based on the following equation:

$\theta^* = T + e/\gamma^*$

where θ^* is an apparent equivalent temperature; T is absolute temperature; e is partial pressure of water vapour in air, expressing water vapour density; γ^* is an apparent value of psychrometer constant defined by:

$\gamma^* = \gamma(r_V/r_H)$

where r_H is the appropriate diffusion resistances for heat transfer by convection, i.e. sensible heat; r_v is the appropriate diffusion resistances for heat transfer by vapour evaporation. γ is a psychrometer constant defined by:

$\gamma = c_p p/\lambda\varepsilon$

where c_p is the specific heat of air at constant pressure; p is the water vapour density; λ is latent heat of vaporisation of water; ε is ratio of molecular weight of water vapour and air (Monteith, 1973).

Figure 16 is a psychrometric chart (Barometric Press -750 mm of Mercury) which gives the complex relations between dry bulb temperature, wet bulb temperature, equivalent temperature, vapour pressure (water vapour density), relative humidity and dew point (see Figure 6, Section 1.6.3, Page 54 for details).

Figure 17 is also a psychrometric chart which is based on the above Basic

programme and gives the complex relations between dry bulb temperature (°C), relative humidity (%) and apparent equivalent temperature (°C).

2.5.3. General data analyses

Data from experiments performed as balanced designs were analysed statistically by one-way and two-way analysis of variance considering treatments groups within each temperature. Differences among least square means were estimated using the Student un-paired t-test for unequal numbers. Statistics were calculated using MINITAB (Minitab Inc., 3081 Enterprise Drive, State College, PA16801, USA), GENSTAT (Genstat 5 Committee, Rothamsted Experimental Station, Harpenden, Hertfordshire, UK, AL5 2JQ) and a Hewlett-Packard General Statistics Pac. (Hewlett-Packard International, 3495 Deer Creek Road, Palo Alto, CA94034, USA).

All results are presented as the means±S.E.M. Values in tables with different letters (superscripts) are significantly different at P less than 0.05 level.

Chapter Three

The endocrine responses in chronic severe heat stressed broiler chicken are not entirely mediated by depressed food intake

3.1. Are the endocrine responses entirely mediated by depressed food intake?

3.1.1. Introduction

It is well established that chronic exposure to high ambient temperatures depresses growth rate in a number of species including broiler chickens (Howlider and Rose, 1987). Despite efforts to improve poultry production in the tropical and subtropical areas of the world, commercial poultry producers still suffer considerable economic loss each year due to irreversible heat prostration. Although the physiological effects of thermal stress in poultry have been reviewed extensively (Smith and Oliver, 1971; Washburn, 1985; Austic, 1985), little is known of the physiological condition which is the exact mechanisms controlling growth rate of heat stressed chickens. Fuller and Dale (1979) suggested that the decline in growth rate does not result entirely from reduced feed intake in heat stressed chickens (see Section 1.8, Page 93-96 for details).

Food intake in chicken is inversely related to ambient temperature (Howlider and Rose, 1987). Reduced food intake is one way to react to elevated high temperature (Mitchell and MacLeod, 1983). Since high temperature exposure causes reduced food intake (Smith and Oliver, 1971) and reduced food intake might link with changed thyroid function (Mitchell and Raza, 1986a) and the peripheral growth hormone (GH) concentration (McMurtry and Johnson, 1988) in studies with fasted birds, it is

necessary to investigate the thyroid functions between heat stressed birds and those which had the same food intake as heat stressed birds but maintained in thermoneutral temperature. The different growth rate between pair-fed and heat stressed groups might result from some impairment of a multitude of hormones and growth factors. However, these studies have not yet been conducted.

Before we begin to investigate the growth rate and thyroid hormonal mechanisms regulated by GH of broiler chickens during heat stress, we must confirm that the decline in growth rate of heat stressed chickens is not entirely due to reduced feed intake as Fuller and Dale (1979) suggested (see Section 1.8, page 93-96 for details).

The objective of the first study was therefore to determine the influence of feed intake on performance of broilers raised in a hot (high ambient temperature of 35 °C) or cool (ambient thermoneutral temperature of 21 °C) environment and to investigate the growth rate and thyroid hormonal mechanisms regulated by GH of broiler chickens during heat stress and those raised at the thermoneutral temperature but pair-fed to the same level as heat stressed birds.

3.1.2. Experimental procedure

Fifteen female broiler chickens were used for the experiment. The chickens were collected from the brooder (29 °C), grouped, tagged, transported and randomly assigned to three groups (N=5) and caged in two climate rooms at the age of two weeks. The same light regime was maintained at 14 hr light and 10 hr darkness. The heat stressed group were maintained in a climate chamber at 35 °C, and the other two groups were maintained in climate chambers at 21 °C for two weeks between 2 and 4 weeks of age. The control and heat stressed groups were fed *ad libitum* while the pair-fed group were limited to the same amount of feed as consumed by the chickens in the

Figure 18. Grouping of birds (n=5) indicating room temperatures with humidities and mode of feeding

Treatment	Environmental condition		Mode of Feeding
	Room temperatures	Relative humidities	
Control	21 °C	45±5%	*Ad libitum* feeding
Pair-feeding	21 °C	45±5%	Fed with the same weight of food as eaten by heat-stressed broilers on previous day
Heat-stress	35 °C	45±5%	*Ad libitum* feeding

hot environment during the experiment. The pair-fed group received amounts of food equal to those consumed by the heat stressed group on the previous day. Half of this amounts of food was fed at 9:00 am the remainder was fed at 17:00 pm to the pair-fed group. The grouping of birds into climate rooms is described in the Figure 18. This was done to compare the growth rate, feed intake and feed conversion ratio of the birds at 21 °C (control and pair-fed groups) and 35 °C (heat stress) in a 14-day feeding and growth monitoring experiment.

Feeding of birds was done every day and weighing every other day at approximately the same time for a period of 14 days (a period of acclimation). Observations were recorded. From the 14 day records of feed intake and weight, the means values of feed intake, body weights and daily weight gain for the three groups of birds were calculated. The percentage of the decreased growth rate between chronic heat stressed broiler chickens and those raised at the ambient thermoneutral temperature but pair-fed to the same level as heat stressed birds and compared to thermoneutral control birds was calculated. The food conversion ratio bar graph and growth rate were plotted.

At the end of the experimental period of 14 days, heparinised blood (2 ml) samples were obtained at 11:00 am from all birds by venipuncture (brachial vein). The blood plasma was prepared by centrifugation at 1500 g for 10 minutes and stored at -20 °C prior to assay for chicken GH, T4 and T3 . Plasma concentration of GH was measured by the method of Goddard *et al.* (1988) using a homologous double antibody radioimmunoassay. Plasma concentrations of T4 and T3 were measured by radioimmunoassay (RIA) using commercially available kits (see Section 2.3.1, Page 114 for details).

3.1.3. Results

3.1.3.1. The reduction of growth rate in chronic severe heat stressed broiler chickens is not entirely due to the decreased food intake

The effects of heat stress and pair-feeding upon body weight gain, daily food intake and feed conversion ratio in female broilers are summarised in Figure 19.

Exposure to the 14-days chronic severe heat stress had pronounced effects on body weight gain, daily food intake and feed conversion ratio. Pair-feeding slightly reduced growth rates ($p<0.05$). Within the 14-day of exposure, heat stress reduced growth rates 22 % more than pair-feeding treatment, although pair-fed and heat stressed birds ate the same amount of food (Figure 19c). Pair-feeding improved feed conversion efficiency (decreased feed conversion ratio, $p<0.05$) while the heat stress treatment reduced feed conversion efficiency (increased feed conversion ratio, $p<0.001$ - Figure 19d) in comparison with thermoneutral control birds.

Although pair-fed and heat stressed birds ate the same amount of food, better growth rate was found in pair-fed birds (Figure 19a). This result confirmed the finding of Fuller and Dale (1979) that decreased food intake was not the only explanation for decreased growth rate in heat stressed birds. This result indicates that growth was depressed by 41% in chickens in the hot environment. However, when "pair-fed" chickens in the cool environment were fed the same amount of feed as consumed by the chickens in the hot environment, their performance was reduced by only 19% compared with chickens fed on an *ad libitum* basis in the cool environment. The results indicated that limiting food intake alone does not result in as severe a reduction in performance as maintaining the birds in a hot environment with the same level of food intake. Clearly, high environmental temperatures impose limitations on

Figure 19. The effect of hot environment and paired feeding in chickens at 21 °C and 35 °C. Values are expressed as means ± SEM for five female broilers.

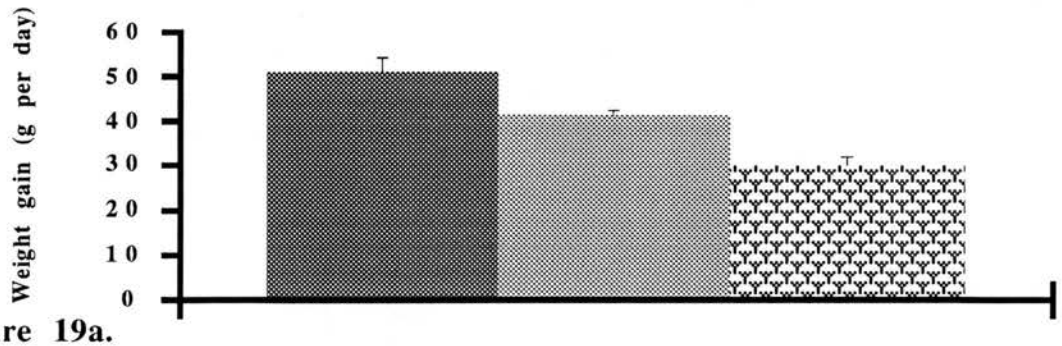


Figure 19a.

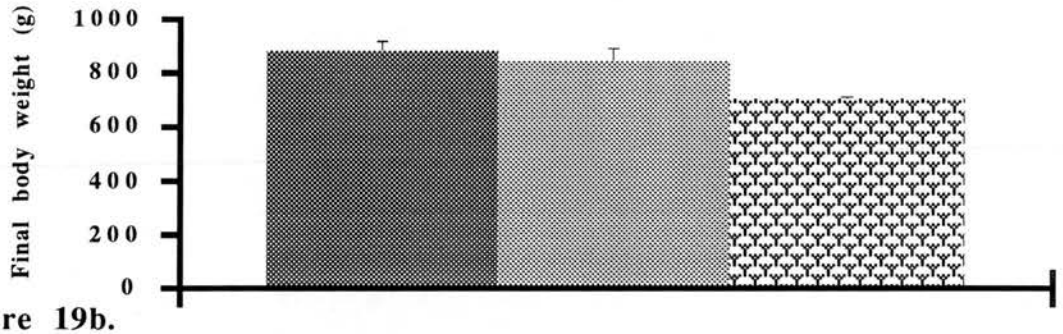


Figure 19b.

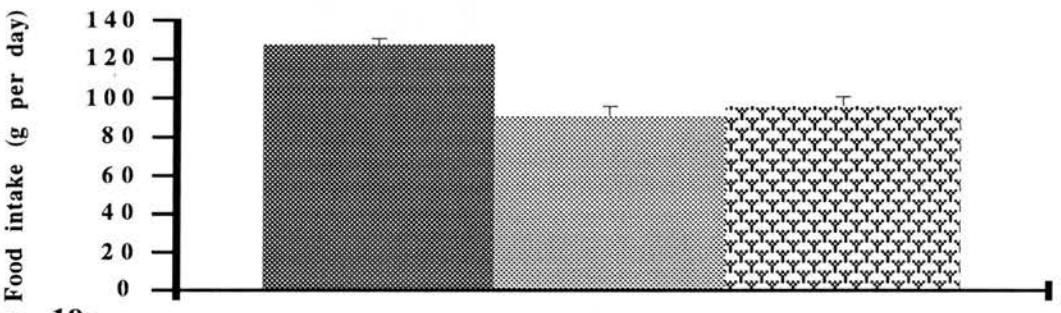


Figure 19c.

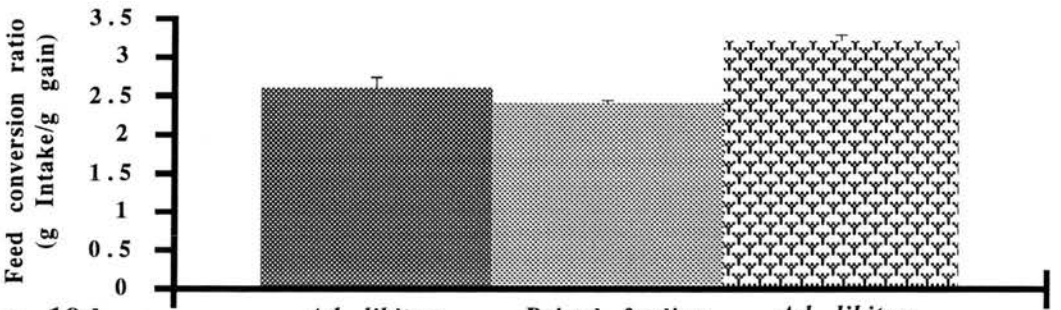


Figure 19d.

Figure 19. The effect of hot environment and paired feeding in chickens at 21 °C and 35 °C. Values are expressed as means ± SEM for five female broilers.

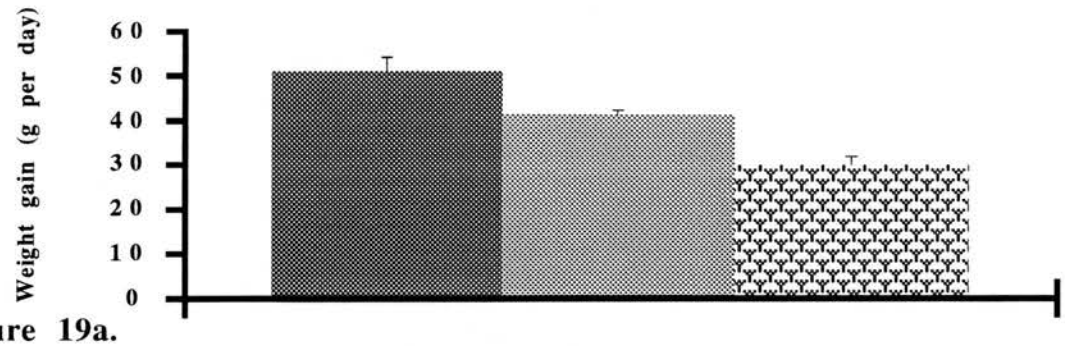


Figure 19a.

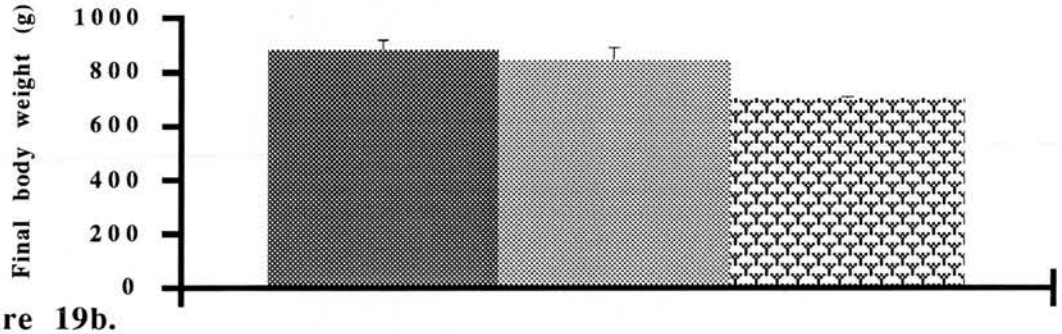


Figure 19b.

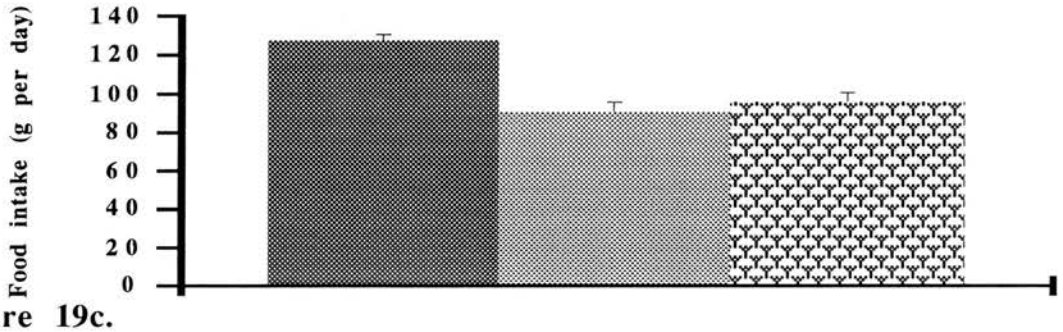


Figure 19c.

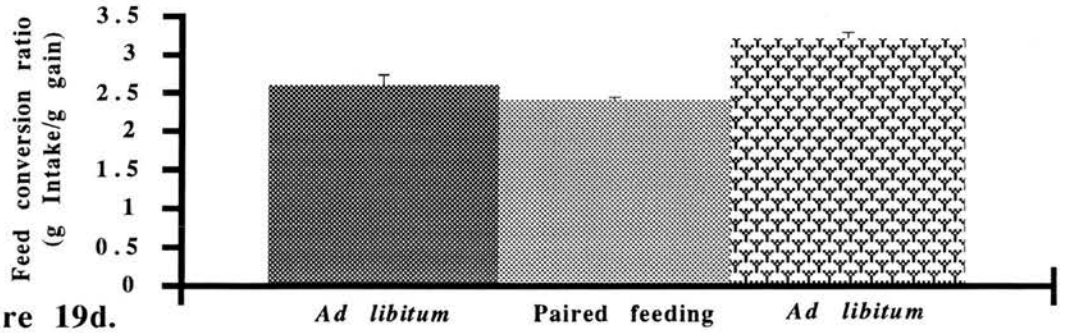


Figure 19d.

Figure 19. The effect of hot environment and paired feeding in chickens at 21 °C and 35 °C. Values are expressed as means ± SEM for five female broilers.

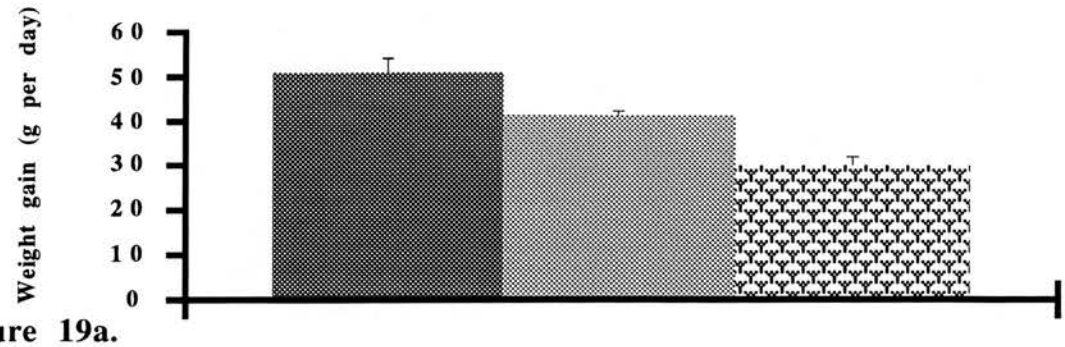


Figure 19a.

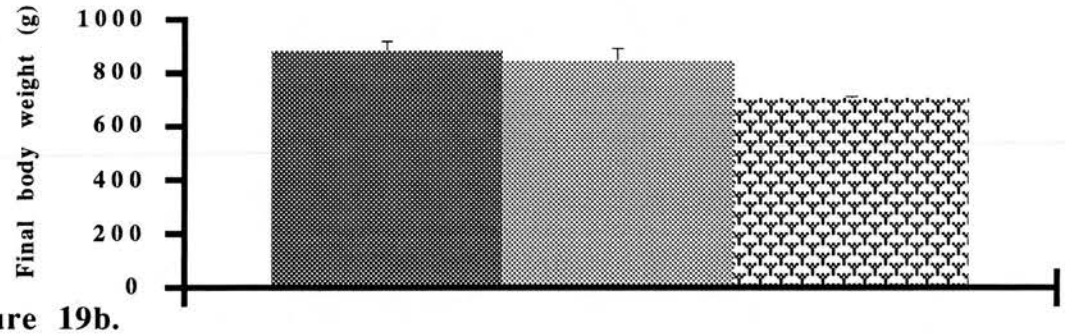


Figure 19b.

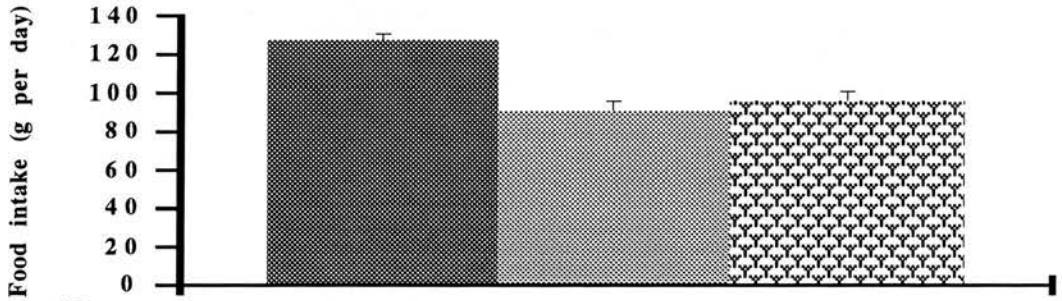


Figure 19c.

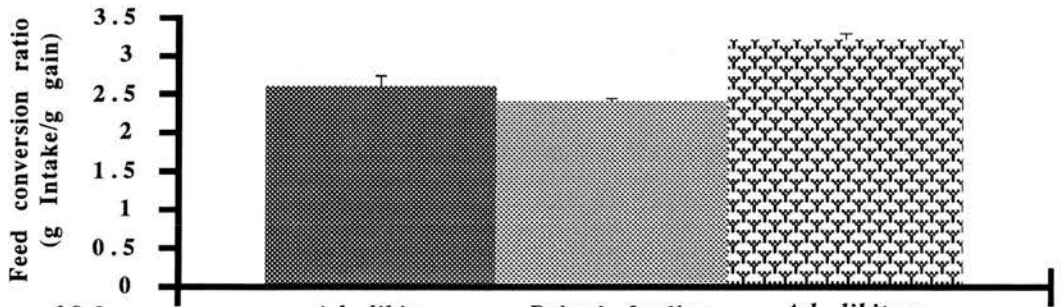


Figure 19d.

Figure 19. The effect of hot environment and paired feeding in chickens at 21 °C and 35 °C. Values are expressed as means ± SEM for five female broilers.

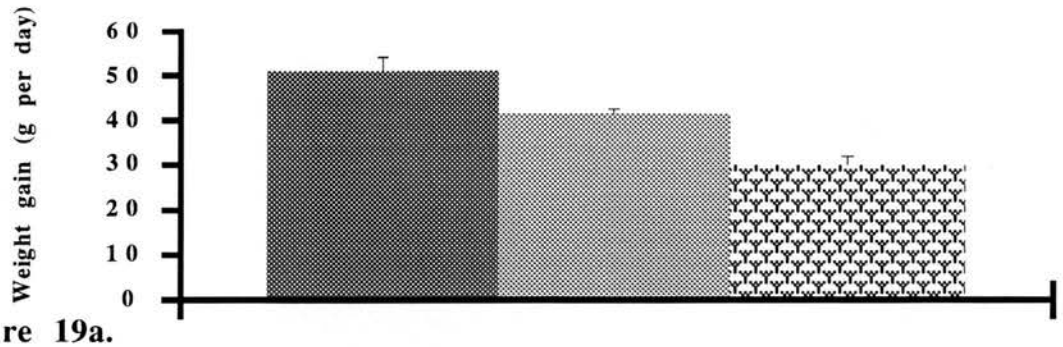


Figure 19a.

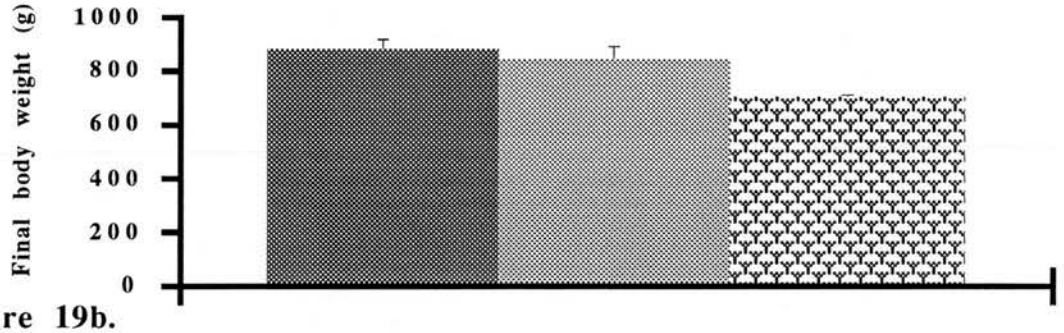


Figure 19b.

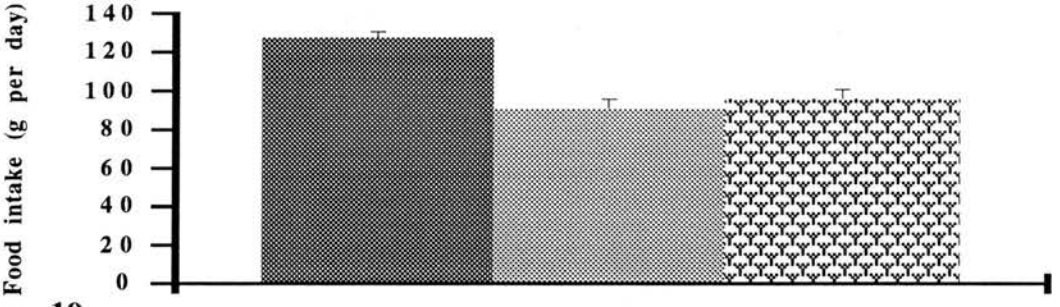


Figure 19c.

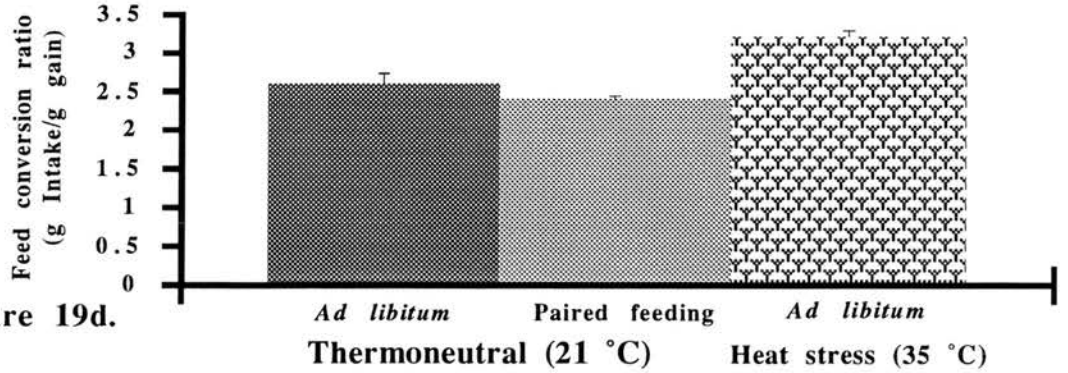


Figure 19d.

Figure 20. Comparison of the baseline plasma hormonal concentration between paired feeding and heat stressed broiler chickens at 21 °C and 35 °C. Values are expressed as means ± SEM, n=5.

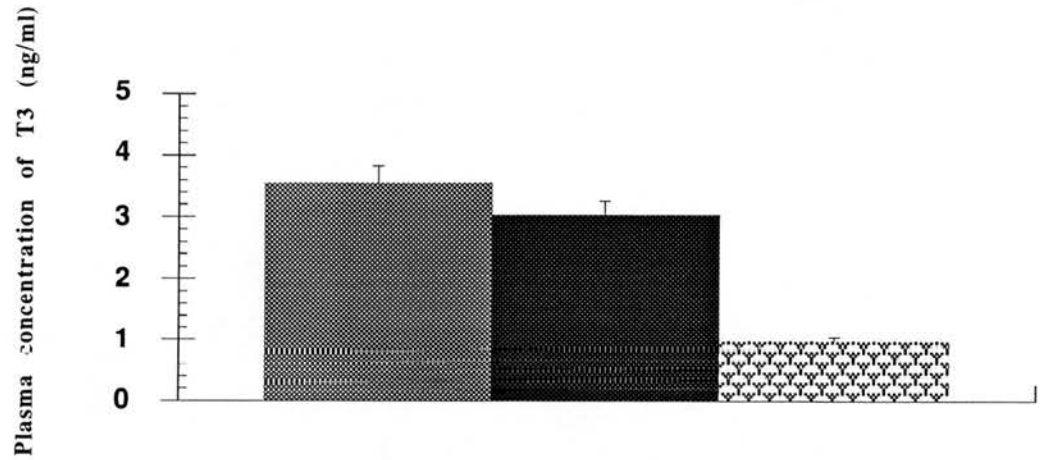


Figure 20a.

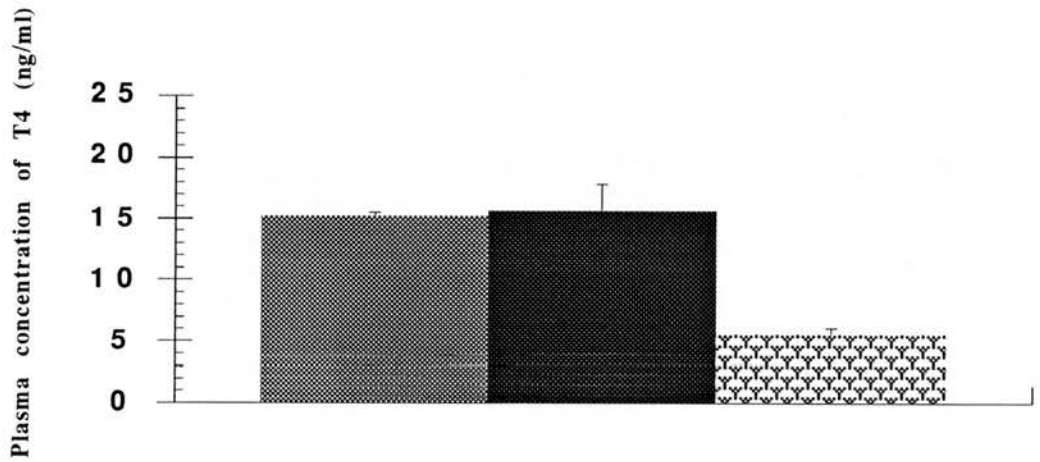


Figure 20b.

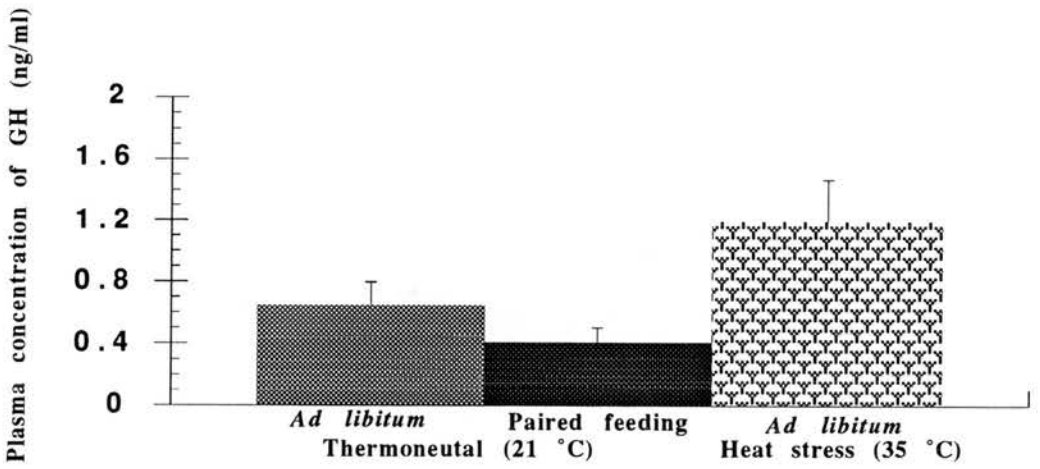


Figure 20c.

Figure 20. Comparison of the baseline plasma hormonal concentration between paired feeding and heat stressed broiler chickens at 21 °C and 35 °C. Values are expressed as means ± SEM, n=5.

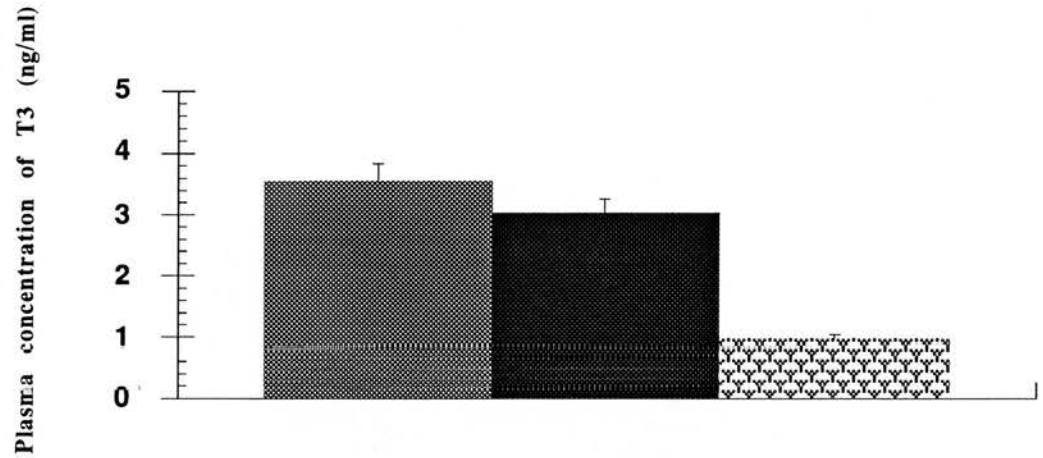


Figure 20a.

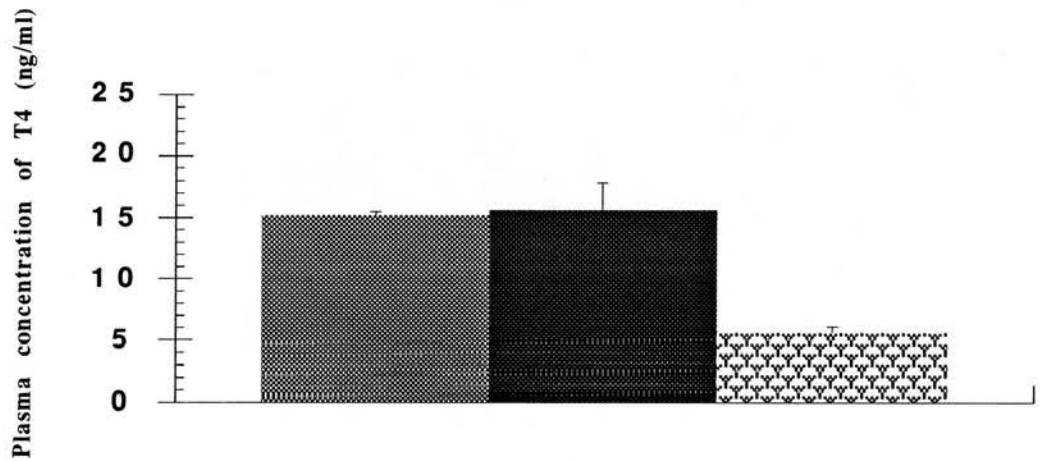


Figure 20b.

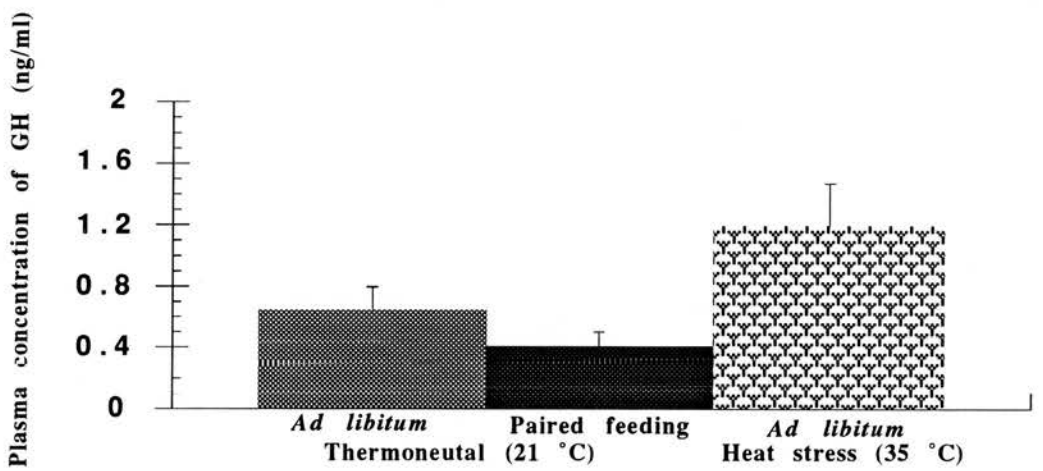


Figure 20c.

the performance of both broilers (Fuller and Dale, 1979) and laying hens (Smith and Oliver, 1972) that are partly unrelated to food intake. These results raised the possibility that reduced food consumption may account for some of the impairment in production (Austic, 1985). It is generally concluded that (a) high temperature exposure (35 °C for 14 days) depresses food intake and growth rate in broilers and reduces feed conversion efficiency (increases feed conversion ratio) and (b) the depression of growth rate is not entirely mediated by the reduction in food intake. It may be due to a direct inhibition of a multitude of hormones secretion and metabolism. The different growth rate between pair-fed and heat stressed groups might result from some of the impairment in a multitude of hormones and growth factors. However, these studies have not yet been conducted.

The results indicated that when assessing the effects of "hot" climates or heat stress upon growth in chickens, reduced food consumption may account for only some of the impairment in production because the pair-fed group which were limited to the same amount of feed as consumed by the chickens in the hot environment during the experiment have better growth performance than the heat stress treatment group.

3.1.3.2. The changes of the peripheral T4, T3 and GH concentrations in chronic severe heat stressed broiler chickens are not due to the decreased food intake

The effects of heat stress and pair-feeding upon the baseline plasma T4, T3 and GH concentrations in female broilers are presented in Figure 20.

Two weeks of heat stress caused a significant ($p<0.05$) reduction in the plasma basal T3 (Figure 20a) and T4 (Figure 20b) concentrations in comparison with the control and the pair-fed birds at 21 °C. No significant differences between the control

Figure 20. Comparison of the baseline plasma hormonal concentration between paired feeding and heat stressed broiler chickens at 21 °C and 35 °C. Values are expressed as means ± SEM, n=5.

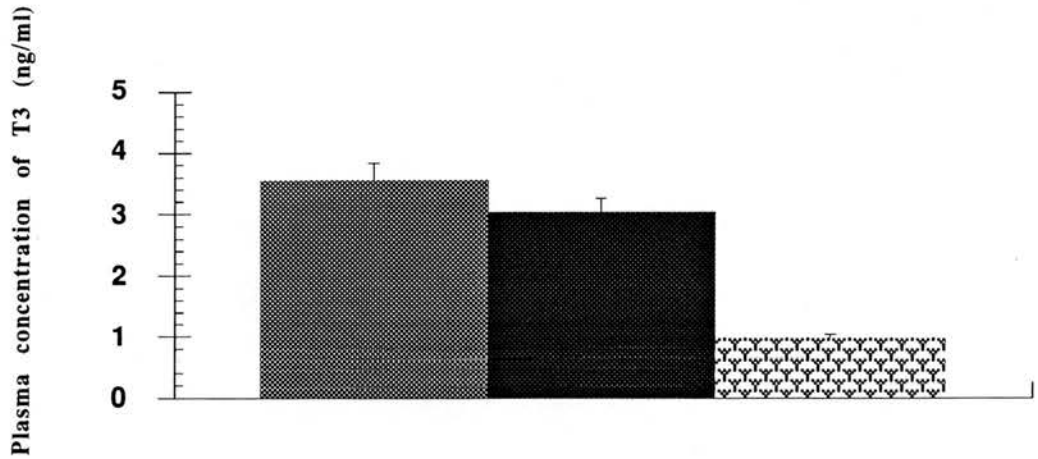


Figure 20a.

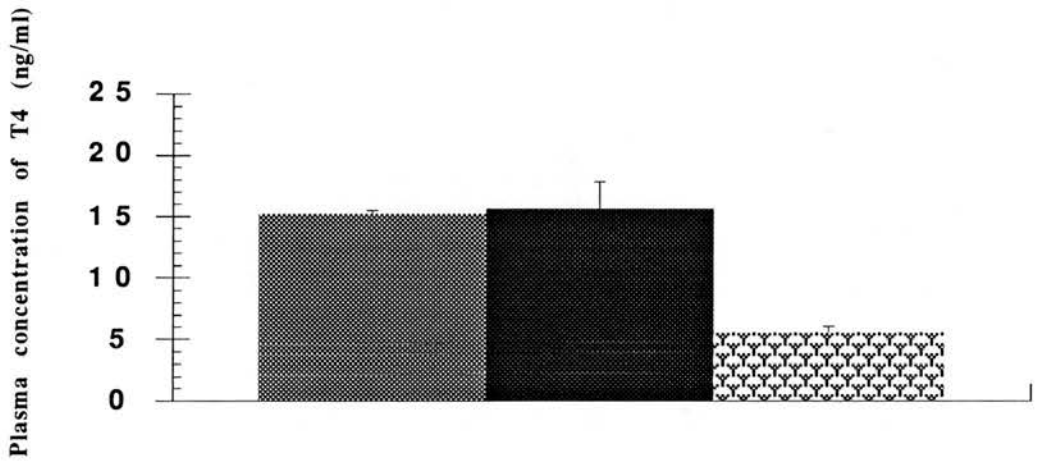


Figure 20b.

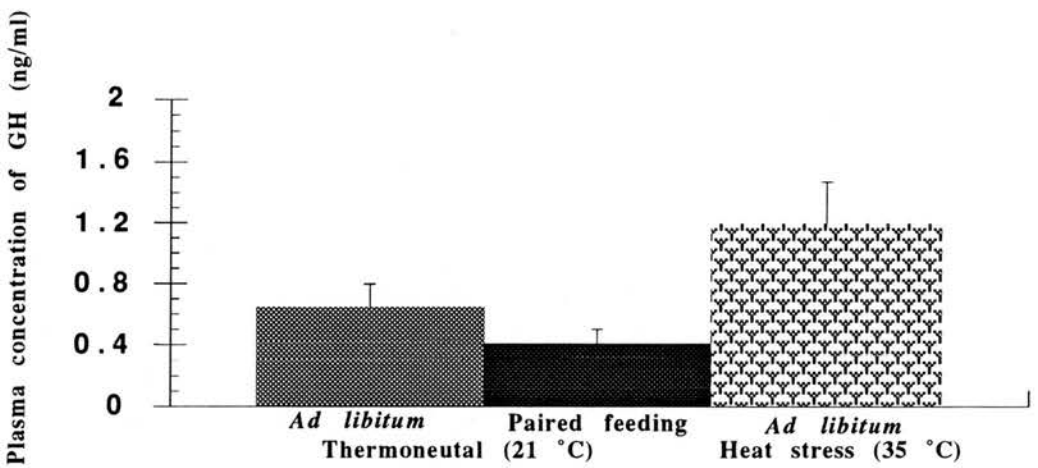


Figure 20c.

Figure 20. Comparison of the baseline plasma hormonal concentration between paired feeding and heat stressed broiler chickens at 21 °C and 35 °C. Values are expressed as means ± SEM, n=5.

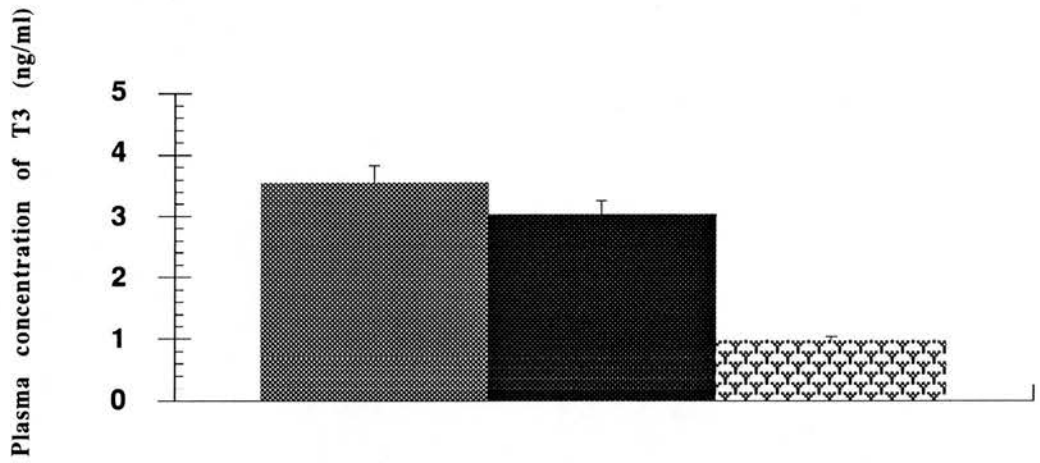


Figure 20a.

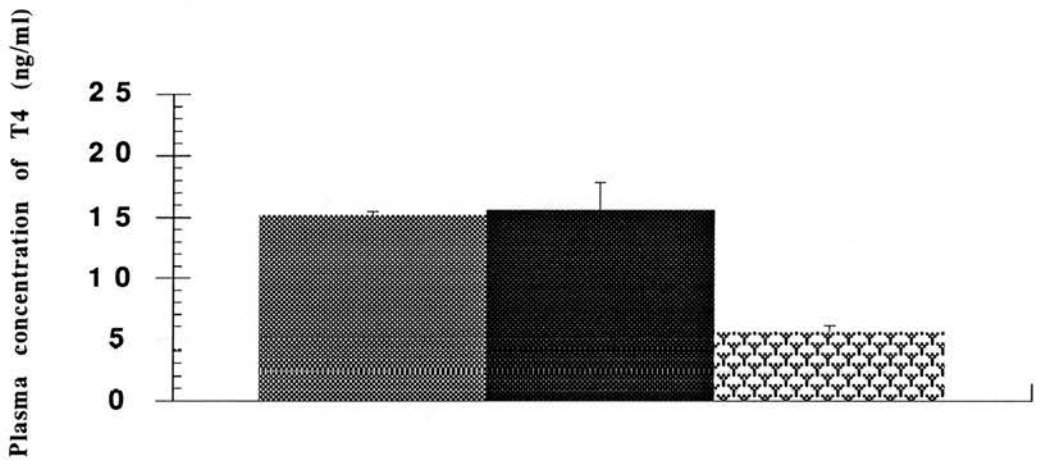


Figure 20b.

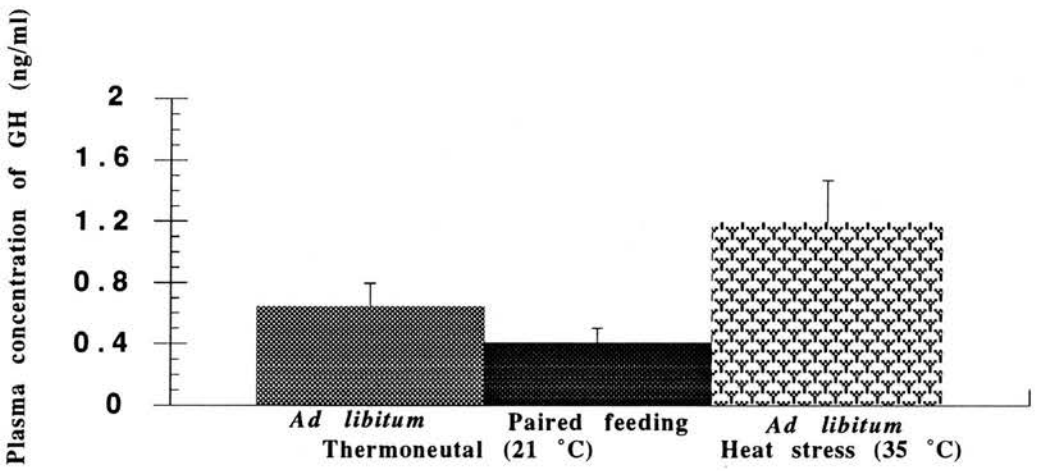


Figure 20c.

and the pair-fed birds at 21 °C were observed in peripheral basal T4 and T3 concentrations. The results indicated that inhibition of thyroid hormones in birds exposed to chronic severe heat stress for two weeks is not due to the decreased food intake. Three fold higher ($p<0.05$) baseline plasma GH concentration (Figure 20c) was found in the heat stressed chickens, than in "pair-fed" chickens in the cool environment. No significant difference between the control and the pair-fed birds at 21 °C was observed in the peripheral basal GH concentrations. Although pair-fed and heat stressed birds ate the same amount of food, better growth rate in pair-fed birds (see Section 3.1.3.1, Page 124 for details) and significant differences of the baseline plasma T4, T3 and GH concentrations between heat stress and pair-feeding were found. The results indicated that limiting food intake alone does not result in increased baseline plasma GH concentration like maintaining the birds in a hot environment with the same level of food intake. This result combined with previous work had indicated that decreased food intake was not the only explanation for decreased growth rate in heat stressed birds. The different growth rates in pair-fed and heat stressed groups might result from higher baseline plasma GH concentration and lower baseline plasma T4 and T3 concentrations.

The results indicated that increased peripheral baseline GH concentration in the chronic severe heat stressed birds was not due to decreased food intake since "pair-fed" chickens did not increase their peripheral baseline GH concentration compared with thermoneutral control birds.

Our finding of long term heat stress reduced plasma T3 concentration agrees with findings from authors cited in Figure 9 (page 84). Plasma T4 was reduced in broilers subject to chronic severe heat stress in agreement with Bobek *et al.* (1980); May (1978); May and McNaughton (1980); Rudas and Pethes (1984); Mitchell and Goddard (1990); Mitchell and Carlisle (1992) but contrary the findings of Bowen and

Washburn (1985); Cogburn and Harrison (1980); Iqbal *et al.* (1987); Moss and Balnave (1978), who reported increased plasma T4 concentration.

3.2. The inhibition of thyroid hormones and the stimulation of GH responses to TRH *in vivo* and the inability of GH to stimulate 5'-monodeiodinase activity in chronic severe heat stressed broiler chickens

3.2.1. Introduction

In the current stage of knowledge it is difficult to explain the confusion in responses in the levels of T4 and T3 in heat stressed chickens. Moss and Balnave (1978) who conducted a similar study using 18-day and 25-day old male chickens (unknown breed), maintained at temperatures of 22 and 30 °C for 16 and 28 days, respectively, and found that the plasma thyroxine concentrations were increased by 56 (16 days) or 103% (28 days), respectively as ambient temperature increased form 22 to 30 °C in birds examined for 16 or 28 days. When birds in the 22 °C environment were limited to the levels of food consumption of the 30 °C group, the plasma thyroxine concentration was not increased but decreased by 12% or was increased by only 50%, respectively in birds examined for 16 or 28 days. The interpretation of the results was, however, difficult as they did not measure T3, and did not monitor ambient humidity, record body weight, or report which strain was used.

The previous studies have demonstrated that the depressed growth rate in chickens exposure to heat stress may result from inhibition of thyroid hormones and not entirely from the decreased food intake. High environmental temperatures impose limitations on the performance of broilers which may be related to changes in the peripheral baseline GH, T4 and T3 concentrations rather than food intake.

The published results of the peripheral baseline T4 and T3 concentrations in heat stressed chickens are, however, not reliable, as conflicting reports exist describing the changes in plasma T4 and T3 concentrations in these birds. It is, therefore, necessary to examine the responses of the hypothalamus, the anterior pituitary gland, the thyroid gland and the 5'-monodeiodination to elevated exogenously administered thyrotrophin releasing hormone (TRH) *in vivo*, in relation to the plasma concentrations of T4, T3 and GH in order to establish if changes in thyroid hormone levels by high environmental temperature are caused by a changed responsiveness of the thyrotrophic-somatrophic axis.

To our knowledge, the influence of high environmental temperature and "pair-fed" treatment on GH pulses or GH responses to TRH is not yet known. However, *in vivo* GH responses in immature broilers to TRH was stimulating 5'-monodeiodination (Scanes *et al.*, 1983). It has been well established that thyrotrophin releasing hormone (TRH) is a potent GH secretagogue in young chickens (Harvey and Scanes, 1984) and it may well be the principal endogenous stimulatory releasing factor in chickens since passive immunisation with anti-TRH suppressed GH secretion (Klandorf *et al.*, 1985).

In order to test the function of the hypothalamic-pituitary-thyroid axis and the thyrotrophic-somatrophic axis and to examine the GH control of 5'-monodeiodination responses to TRH *in vivo* in heat stressed broilers (Mitchell and Goddard, 1990), it was therefore considered appropriate to study plasma T4, T3 and GH responses to exogenously administered TRH *in vivo* during chronic heat stress and "pair-fed" treatment in growing broiler chickens between 2 and 4 weeks of age.

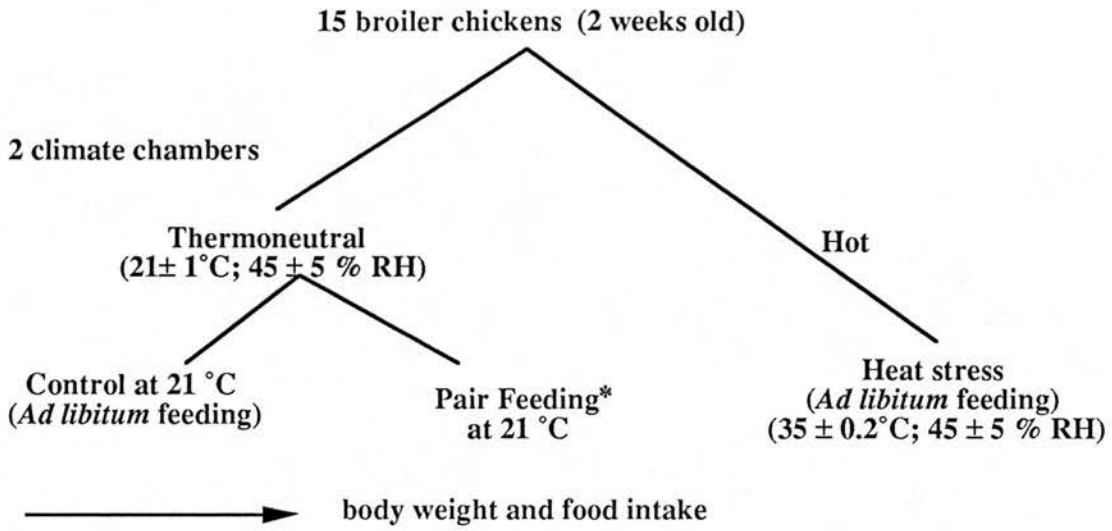
3.2.2. Experimental procedure

Fifteen female broiler chickens described in Section 3.1.2 (page 122), were used for the experiment. The broilers were collected from brooder, grouped, tagged and caged in two climate rooms at the age of two weeks. The two climate rooms were used and maintained at two different temperatures, 21 °C (cool environment for control and pair-fed groups) and 35 °C (hot environment for heat stressed groups). The same light intensity was maintained at 14 hr light and 10 hr darkness. Chickens were fed *ad libitum* in both environments, and an additional group of "pair-fed" chickens in the cool environment were limited to the same amount of food equal to those consumed by the heat stressed group on the previous day. Half of this amounts of food was fed at 9:00 am the remainder was fed at 17:00 pm to the pair-fed group. The grouping of birds into climate rooms is described on Figure 21. This was done to compare the growth rate, feed intake and feed conversion ratio of the birds at 21 °C (control and pair-fed groups) and 35 °C (heat stress) in a 14-day feeding and growth monitoring experiment.

Feeding of birds was done every day and weighing every other day at approximately the same time for a period of 14 days (a period of acclimation). Observations were recorded. From the 14 day record of feed intake and weights obtained, the means values of feed intake, body weights and daily weight gain for the three groups of birds were calculated. The percentage of the decreased growth rate between chronic heat stressed broiler chickens and those raised at the ambient thermoneutral temperature but pair-fed to the same level as heat stressed birds and compared to thermoneutral control birds was calculated.

At the end of the experimental period of 14 days, heparinised blood (2 ml) samples were obtained at 11:00 am from all birds by venipuncture (brachial vein)

Figure 21. Materials and Methods



Note: * Pair Feeding = The birds in Pair-Fed group were fed with the same amount of food as eaten by heat stressed birds on previous day.

4 weeks of age

Blood samples (0)

Inject TRH (s.c.) 10 μg kg^{-1}

Blood samples at 40, 80, 120 and 160 minutes post injection

before they received a subcutaneous injection of TRH (10 μg/kg body weight) to test the stimulation of pituitary GH secretion and its role in the regulation of thyroid hormonal mechanisms since synthetic TRH is a potent thyrotrophin and prolactin secretagogue in mammals and birds. In addition, TRH can stimulate GH secretion under some circumstances in domestic fowl (Scanes *et al.*, 1977) and duck (Pethes *et al.*, 1979). Then, four further samples were taken at 40 minutes intervals post-injection. The blood plasma was prepared by centrifugation at 1500 g for 10 minutes and stored at -20 °C prior to assay for chicken GH, T4 and T3. Plasma concentration of GH was measured by the method of Goddard *et al.* (1988) using a homologous double antibody radioimmunoassay. Plasma concentrations of T4 and T3 were measured by radioimmunoassay (RIA) using commercially available kits (see Section 2.3.1, Page 114 for details).

3.2.3. Results

The effects of chronic severe heat stress and "pair-fed" treatment on the concentrations of GH, T4 and T3 in the plasma of the chickens in the experiment and their subsequent responses to TRH are summarised in figures 22 and 23.

Two weeks of heat stress caused a significant reduction in the plasma basal T3 and T4 concentrations (figure 22a and 22b). In the control and the pair-fed group at 21 °C, 10 μg TRH/kg body weight were effective in significantly ($p<0.05$) increasing plasma levels of T4 (Figure 22b) and T3 (Figure 22a) post-injection at 40 and 80 minutes respectively. In heat stress there were significantly lower plasma peaks of T4 (Figure 22b) and T3 (Figure 22a) concentrations compared to pair-fed birds. The inhibition of thyroid hormones and their responses to TRH after chronic severe heat stress in broiler chickens is not due to the decreased food intake. The comparisons of the baseline plasma GH concentration and its subsequent response to TRH between pair-fed at 21 °C and heat stressed broiler chickens at 35 °C are presented in Figure

Figure 22. The effect of paired feeding at 21 °C and heat stress on broiler chickens release of peripheral hormonal concentration (ng/ml) responses to TRH subcutaneous injections (10 μg/kg body weight). Values are expressed as means ± SEM for five female broiler chickens.

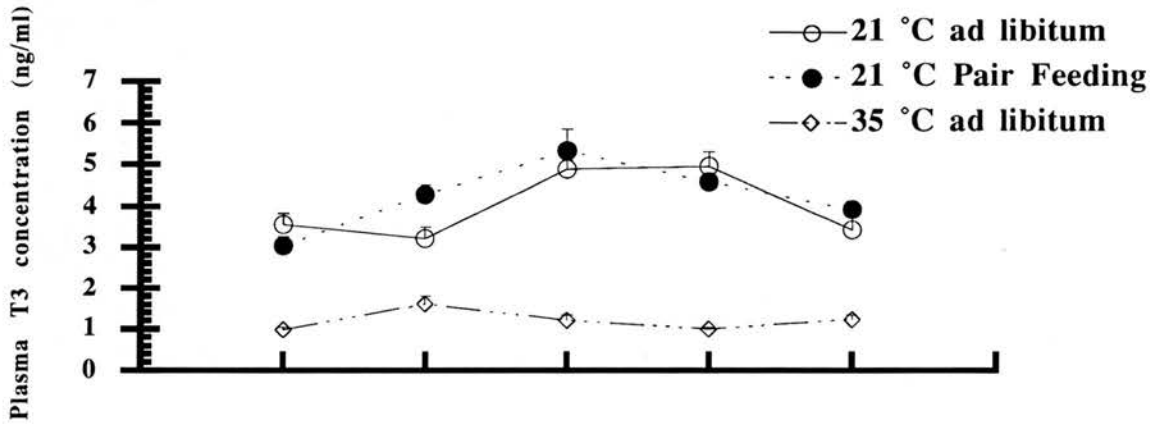


Figure 22a.

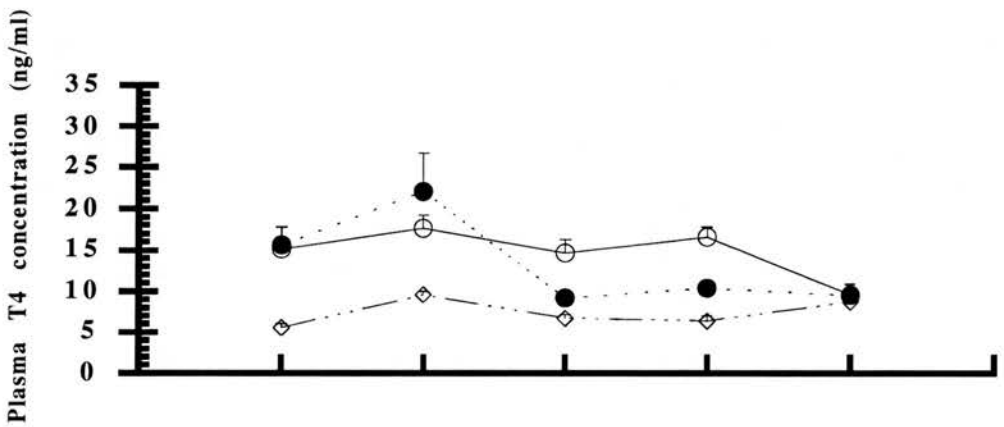


Figure 22b.

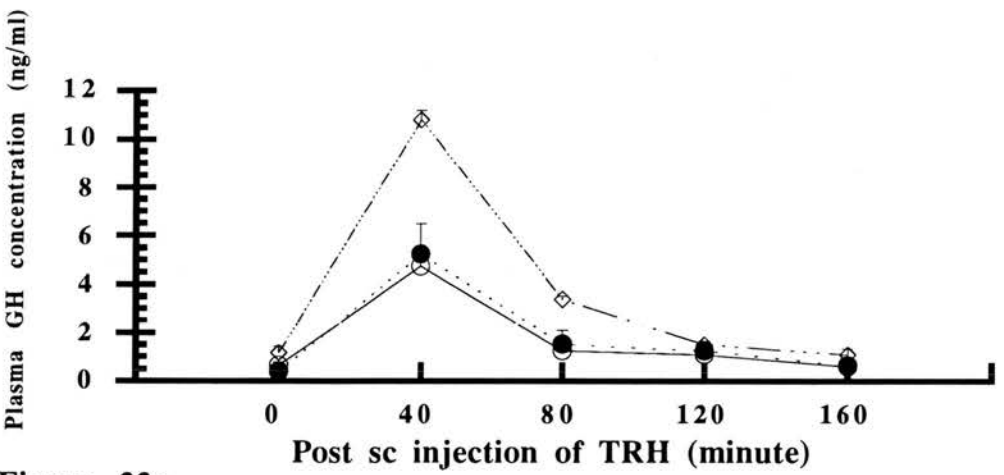


Figure 22c.

Figure 23. Comparison of (a) percentage of daily food intake (%), (b) percentage of daily weight gain (%), (c) percentage of feed conversion ratio (%) of broiler chickens at 21 °C and 35 °C.

Description	Thermoneutral environment		Hot environment
	Control birds were fed *ad libitum*	Pair fed birds	Heat stress birds were fed *ad libitum*
Ambient temperature	21 °C	21 °C	35 °C
Daily food intake (%)	100^{a} (127.6±3.3094)	71^{b} (90.7±5.4113)	75^{b} (95.8±4.8746)
Weight gain (%)	100^{a} (50.7±3.3094)	81^{b} (41.2±1.118)	59^{c} (29.9±2.1019)
Feed conversion ratio (%)	100^{b} (2.6±0.1342)	92^{c} (2.4±0.0447)	123^{a} (3.2±0.0894)

Percentage of daily values are presented as the percentage of control birds at 21 °C for pair fed birds at 21 °C and birds at 35 °C (N=5).

Values within line with different letters (superscripts) are significantly different at least than 0.05% level.

A higher value for feed conversion ratio is an index of poor feed conversion efficiency.

22c. Stimulation of peripheral GH production by TRH is increased significantly ($p<0.05$ - Figure 22c) by heat stress in comparison with those thermoneutral broilers pair-fed to heat stressed birds. No significant differences between the control and the pair-fed birds at 21 °C were observed in the peripheral basal GH, T4 and T3 concentrations and their subsequent responses to TRH. The results indicated that inhibition of thyroid hormones and greater stimulation of peripheral GH production by TRH in birds during exposure to chronic severe heat stress for two weeks was not due to decreased food intake.

Although pair-fed and heat stressed birds ate the same amount of food, inhibition of thyroid hormones and higher stimulation of peripheral GH production by TRH was found in the heat stressed group compared to the pair-fed birds (Figure 22c). The results indicate that depressed growth rate by 41% (Figure 23) in chickens in the hot environment was accompanied by a three fold higher plasma GH concentration (Figure 22c) compared to "pair-fed" chickens in the cool environment. The results indicated that limiting food intake alone does not result in increased stimulation of peripheral GH production by TRH.

The different growth rate between pair-fed and heat stressed groups might result from lower metabolic clearance of GH per unit of metabolic weight and lower plasma T4 and T3 concentrations. It has been shown previously that the peripheral production of T3 may depend on a functional TRH-GH axis, since both TRH and GH are capable of stimulating hepatic 5'-monodeiodinating activity (Mitchell and Raza, 1986a, 1988; Kühn *et al.*, 1986). In the current stage of knowledge it is difficult to explain the unresponsiveness of 5'-monodeiodination to higher GH in heat stressed birds. However, It has been shown that in chickens possessing the sex-linked dwarf gene whose growth rate are depressed and plasma T3 levels are reduced (Lam *et al.*, 1989) despite elevated GH concentrations, apparent dissociation of GH from control

of 5'-monodeiodination were attributed to a decreased capacity of GH receptors (Kühn *et al.*, 1989). It is therefore possible that during heat stress, the GH receptors become non-functional for GH stimulation or the number of GH receptors may be reduced, and a lack of TSH-response could explain the lower T4 while a lack of GH-response could be responsible for the unresponsiveness of 5'-monodeiodination. The inability of GH to stimulate 5'-monodeiodinase activity in chronic severe heat stressed broiler chickens is also not due to the decreased food intake

3.3. Hyperthermia increases the peripheral GH half life

3.3.1. Introduction

When prevailing temperatures rise above the zone of thermoneutrality, a reduction in feed intake of broiler chickens can be expected to occur, and this is usually accompanied by a decline in growth rate. The previous work had indicated that decreased food intake was not the only explanation for decreased growth rate in heat stressed birds. These results raised the possibility that higher baseline plasma GH concentration, longer peripheral GH half life and higher stimulation of peripheral GH production by TRH may account for some of the impairment in production. The different growth rates in pair-fed and heat stressed groups might result from some of the impairment in a multitude of hormones and growth factors (see Section 1.4, page 20 for details).

The previous studies have demonstrated that the depressed growth rate in chickens exposed to heat stress conditions may result from inhibition of thyroid hormones despite elevated GH concentration and its responses to exogenously administered TRH *in vivo*, apparent dissociation of GH from control of 5'-monodeiodination, and not entirely due to the decreased food intake.

The different growth rates in pair-fed and heat stressed groups might result from higher baseline plasma GH concentration and higher stimulation of peripheral GH production by TRH. It is, however, not known whether the higher GH level is due to higher GH secretion rate or due to slower GH degradative rate (longer peripheral GH half life) in chronic severe heat stressed chickens. The responses of the 5'-monodeiodination to elevated exogenously administered chicken growth hormone (cGH) *in vivo* have, therefore, been examined, in terms of the effects on plasma concentrations of T4, T3 and GH in order to establish if changes in thyroid hormones and growth hormone levels by high environmental temperature or "pair-fed" treatment is caused by a changed interaction of the half life of GH and its control of 5'-monodeiodination.

GH in broiler-strain chickens is secreted in a pulsatile manner, with peaks of consistent frequency and duration. The pulsatile secretory pattern characteristics of circulating GH are very important for tending to reflect growth in poultry (Vasilatos-Younken *et al.*, 1988b). The plasma T3 and GH concentrations are also affected by age. To our knowledge, the reason for higher stimulation of peripheral GH responses to TRH in birds influenced by high environmental temperature rather than "pair-fed" treatment, is not yet known. Since temperature changes thyroid hormone levels, a changed 5'-monodeiodination response to GH in the liver might exist.

In order to test the half life of GH and its function of control of 5'-monodeiodination *in vivo* in female broilers, we carried out this experiment to investigate the effects of high environmental temperature and "pair-fed" treatment on the half life of GH and its function of control of 5'-monodeiodination *in vivo*. The objectives of the current study were therefore to investigate the peripheral GH, T3 and T4 concentrations and their responses to exogenously administered cGH (15μg/kg body weight) *in vivo* associated with growth rate between heat stressed chickens and

"pair-fed" chickens that maintained at a thermoneutral temperature. In addition to identify the conflicting results relating to T4 and T3 responses in heat stressed chickens, which might be caused by different ages, older birds were used.

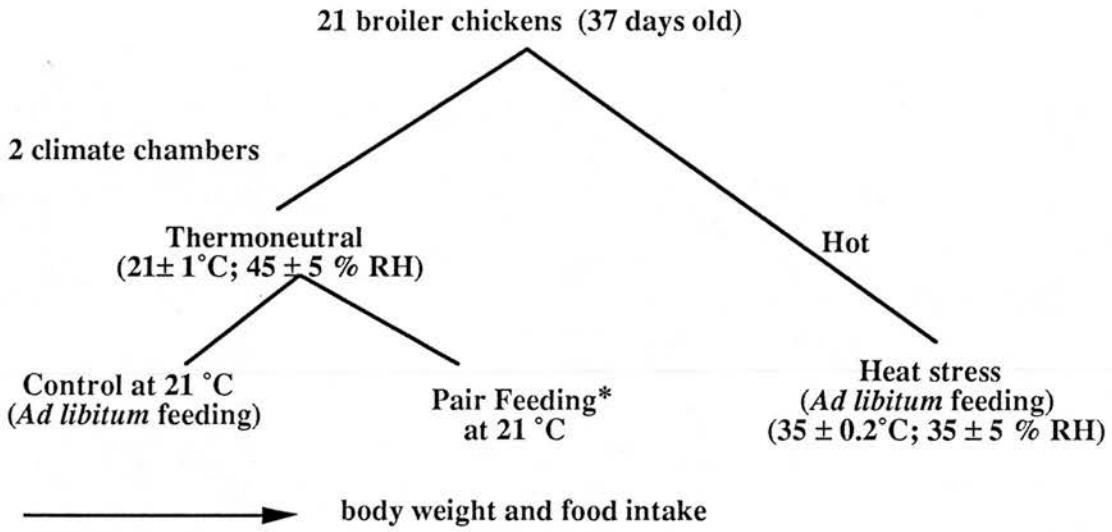
3.3.2. Experimental procedure

Commercial female broiler chickens (Ross Poultry (GB.) Ltd) between age 5 to 7 weeks were used. They were maintained in brooder until 20 days old and vaccinated for Gumboro at age of 7 days.

The female broiler chickens were transported from the poultry house (21 ± 1 °C) to one climate chamber at 21±1 °C with 45 ± % RH and maintained on 14 hr light/day until 35 days of age. Two days after (at age of 37 days), they were randomly assigned to three groups of seven. The heat stressed group were maintained in a climate chamber at 35 ±0.2 °C with 35 ± 5 % RH, and other two groups were maintained in a climate chamber at 21±1 °C with 45 ± % RH on 14 hr light/day, until 51 days of age. The control and heat stressed groups were fed *ad libitum* while the pair-fed group were fed by food restriction (pair-fed to the same level as heat stressed birds) during the experiment. The grouping of birds into climate rooms is described in Figure 24. Food intakes and body weights were measured daily and growth rate and feed conversion ratio calculated. The data for growth performance was standardised and expressed by percentage in order to make the data comparable with other experiments using birds with different ages.

At the end of the experimental period, heparinised blood (2 ml) samples were obtained from all birds by venipuncture before a subcutaneous GH injection (15 μg/kg body weight) at 11:00 am. Then, three further samples were taken at 40 minutes intervals. The blood plasma was prepared by centrifugation at 1500 g for 10 minutes and stored at -20 °C prior to assay for chicken GH, T4 and T3. Plasma concentrations

Figure 24. Materials and Methods



Note: * Pair Feeding = The birds in Pair-Fed group were fed with the same amount of food as eaten by heat stressed birds on previous day.

51 days of age

Blood samples (0)

Inject GH (s.c.) 15 μg kg^{-1}

Blood samples at 40, 80, and 120 minutes post injection

of GH were measured by the enzyme-linked immunosorbent assay (ELISA) described on page 115 (Section 2.3.2). Plasma concentrations of T4 and T3 were measured by radioimmunoassay (RIA) using commercially available kits (see Section 2.3.1, Page 114 for details).

3.3.3. Results

The 14-day feeding and growth monitoring experiment results are summarised in figures 25 - 27.

Two weeks of heat stress caused a significant reduction in the plasma basal T3 (Figure 25a) and T4 (Figure 25b) concentrations. No significant effects of ambient high temperature were observed on the peripheral basal GH concentrations (Figure 25c). 40 minutes after a subcutaneous (sc) injection of chicken GH, elevated plasma GH concentrations in all birds in comparison with basal GH concentrations indicate that all birds received a subcutaneous injection of GH successfully (Figure 25c). After injection of chicken GH, elevated plasma GH concentrations in heat stressed birds were found in comparison with pair-fed birds (Figure 25c). An elevation of the plasma GH concentration in heat stressed birds may be related to a lower disappearance rate of GH or utilising somatotrophin slower because of lower metabolic clearance of GH per unit of metabolic weight respectively. The result indicate that depressed growth rate in chickens in the hot environment was caused by longer peripheral GH half life in comparison with pair-fed chickens in the cool environment.

Exposure to the 14-days chronic severe heat stress had a pronounced effect on body weight gain, daily food intake, the body temperature and feed conversion ratio (Figures 26 and 27). Whilst pair-feeding slightly reduced growth rates ($p<0.05$). Within the 14-day of exposure, heat stress reduced growth rates 41 % more than pair-feeding treatment, although pair-fed and heat stressed birds ate the same amount of

Figure 25. Plasma hormonal concentration (ng/ml) following subcutaneous injections of avian GH (15 μg/kg body weight) to 7-week old chickens. Values are expressed as means ± SEM.

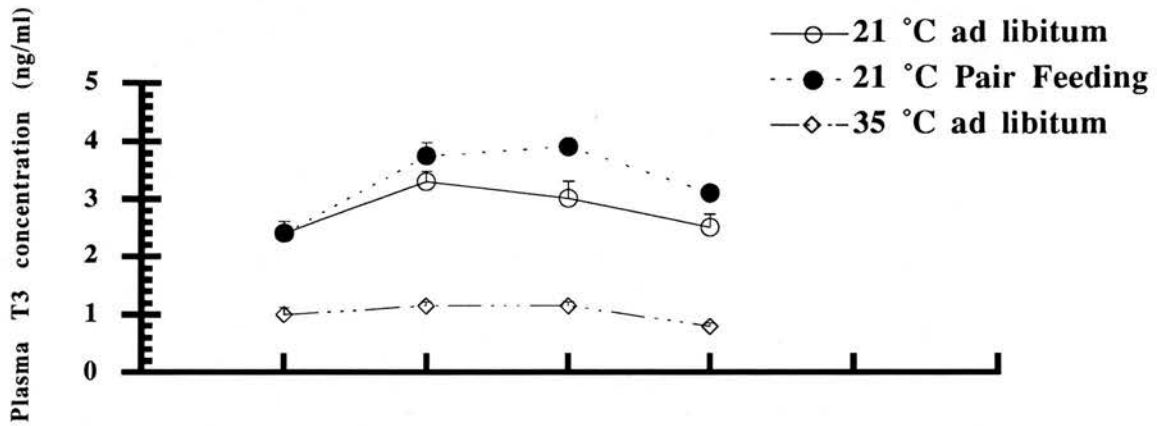


Figure 25a.

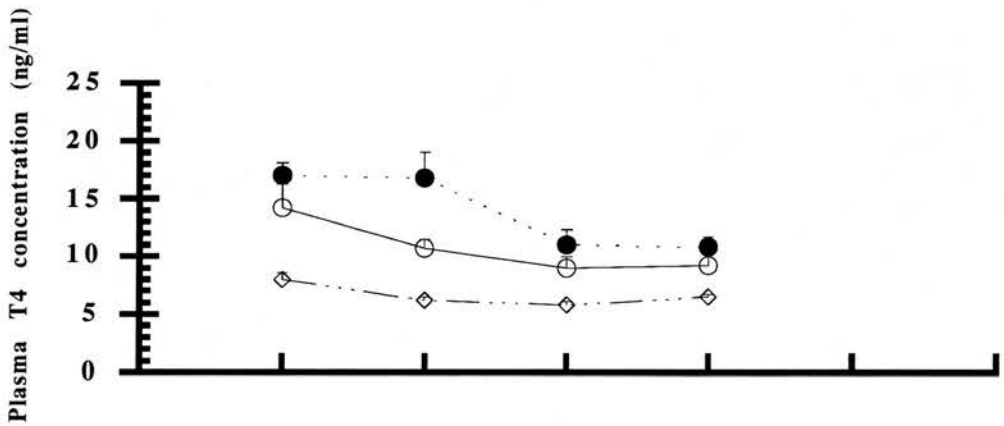


Figure 25b.

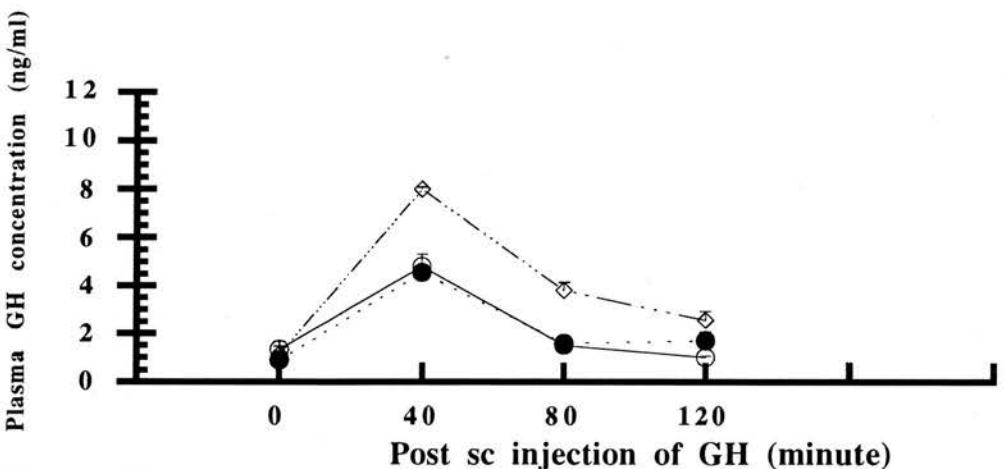


Figure 25c.

Figure 26. Comparison of (a) percentage of daily food intake (%), (b) percentage of daily weight gain (%), (c) percentage of feed conversion ratio (%) of broiler chickens at 21 °C and 35 °C.

Description	Thermoneutral environment		Hot environment
	Control birds were fed *ad libitum*	Pair fed birds	Heat stress birds were fed *ad libitum*
Ambient temperature	21 °C	21 °C	35 °C
Daily food intake (%)	100^{a} (129.69±13.533)	72^{b} (93.4±3.239)	71^{b} (91.31±2.983)
Weight gain (%)	100^{a} (65.01±6.192)	87^{b} (56.51±2.738)	46^{c} (29.94±2.812)
Feed conversion ratio (%)	100^{b} (1.99±0.038)	84^{c} (1.67±0.081)	153^{a} (3.05±0.788)

Percentage of daily values are presented as the percentage of control birds at 21 °C for pair fed birds at 21 °C and birds at 35 °C (N=7).

Values within line with different letters (superscripts) are significantly different at least than 0.05% level.

A higher value for feed conversion ratio is an index of poor feed conversion efficiency.

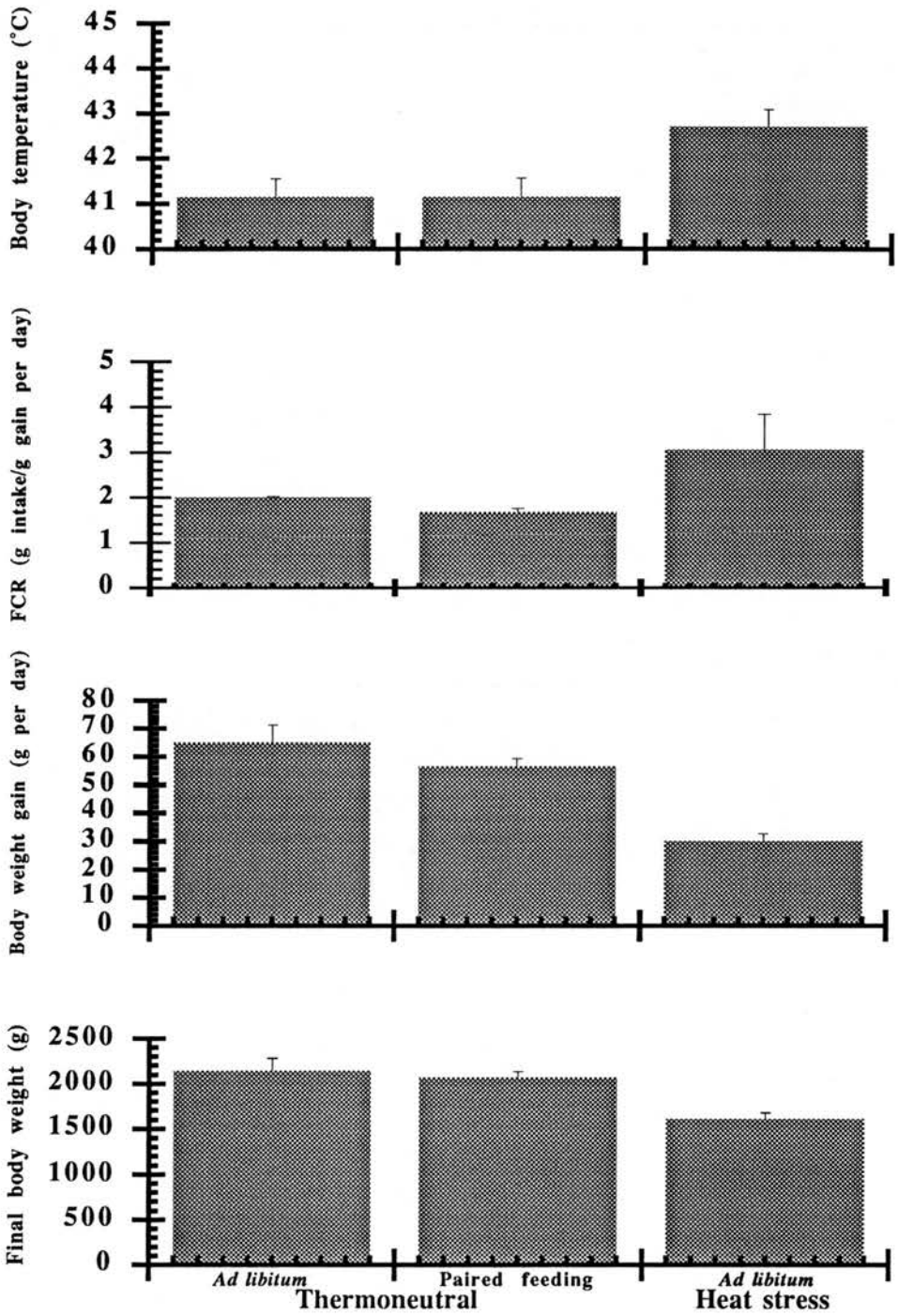


Figure 27. Comparison of body weight (g), body weight gain (g/day), FCR (%) and body temperature (°C) between pair-fed and heat stressed chickens. Values are expressed as means ± SEM, n=7.

$$\text{FCR = Food conversion ratio} = \frac{\text{Food intake (g/d)}}{\text{Body weight gain (g/d)}} = \frac{1}{\text{FCE}}$$

$$\text{FCE = Food conversion efficiency (\%)} = \frac{\text{Body weight gain (g/d) x 100\%}}{\text{Food intake (g/d)}}$$

food ($p<0.001$ - Figure 26). Pair-feeding improved feed conversion efficiency (decreased feed conversion ratio, $p<0.05$) while the heat stress treatment reduced feed conversion efficiency (increased feed conversion ratio, $p<0.001$ - Figure 27) in comparison with thermoneutral control birds.

The result indicate that depressed growth rate (Figure 26) in chickens in the hot environment might be caused by longer peripheral GH half life (Figure 25c) in comparison with "pair-fed" chickens. The results indicate that limiting food intake alone does not result in increased peripheral GH half life like maintaining the birds in a hot environment with the same level of food intake. This result combined with previous work had indicated that decreased food intake was not the only explanation for decreased growth rate in heat stressed birds. The different growth rate between pair-fed and heat stressed groups might result from longer peripheral GH half life and higher stimulation of peripheral GH production by TRH.

The 14-day chronic severe heat stress treatments had a pronounced effect on the growth hormone's function in the control of thyroid hormones metabolism and the regulation of 5'-monodeiodination response to GH, but not pair-fed birds (Figure 25). The T3 did not respond to GH in severe heat stressed birds while increased T3 concentrations were improved significantly over those of baseline responses to GH ($p<0.05$ - Figure 25a) in pair-fed and control birds. The T4 response to GH was greatly decreased in pair-fed and control birds but was unaltered by the chronic severe heat stress treatments despite inhibited absolute concentrations (Figure 25b). These effects were accompanied by a 180 % increase ($p<0.001$) in the GH induced stimulation of GH concentration at 40 minutes after GH injection (Figure 25c).

3.4. Discussion

These results indicated that the decreased growth rate in chronic severe heat stressed broiler chicken was accompanied by changes in endocrine function. Heat stress inhibited the regulation of 5'-monodeiodination response by GH, but this did not occur in pair-fed birds.

These results also suggested that stimulation of peripheral T3 production by TRH is inhibited in heat stressed birds rather than pair-fed birds despite an increased GH response, although pair-fed and heat stressed birds ate the same amount of food. The apparent discrepancy in the relationship between circulating GH concentrations versus growth for the chronic heat stressed birds likely results from a defect in tissue sensitivity to GH, rather than reflecting the true relationship of GH to growth in poultry. Hepatic GH binding might be lower in the chronic heat stressed birds compared with control broilers. Although chronic heat stress condition by itself hardly showed any effect on pre-injection GH values, chronic severe heat stress conditions enhanced the 40-minute post-injection GH values response to GH injection. These results suggested that heat stressed birds rather than pair-fed birds inhibited the GH clearance. A deficiency of hepatic GH receptors in the chronic heat stressed birds was supported by the failure of GH to increase plasma T3 concentration. The increased magnitude of GH peak following GH administration at the chronic severe heat stress conditions may be explicable in terms of the dissociation of GH from control of 5'-monodeiodination, or the depressive effects of thyroid hormones on GH secretion (Harvey, 1983; Harvey, 1990b, Harvey *et al.*, 1991a) while the thyroid function is known to be changed in warmer environments. The changed thyroid function may then result in an enhanced sensitivity of GH to a secretagogue challenge such as TRH (Harvey, 1990c). The enhanced 40-minute post-injection GH values following GH injection in the chronic heat stressed birds should be interpreted carefully.

It may be interpreted as a slower utilisation of somatotrophin and thus an increased half-life of GH in chronic heat stress. From the results in pigs selected for heavier body weights at a given age which have a higher rate of metabolic clearance of somatotrophin (Arbona *et al.*, 1988), and young, growing chickens which have a higher metabolic clearance of GH per unit of metabolic weight than adults (Lauterio and Scanes, 1988), the first possibility seems valid. It has been suggested that animals utilising somatotrophin faster will exhibit lower circulating somatotrophin levels (Siers and Swiger, 1971). Nevertheless, the paradox of a slower utilisation of GH in association with faster growth or larger body size when comparisons are made between the thermoneutral pair-fed birds and heat stressed birds may be unique to species including poultry and pigs for which a large growth-promoting effect of GH has been demonstrated.

The second possibility is rather unlikely in view of the existing theory that GH has a number of well-defined functions including a central role in the control of growth and development. Otherwise this result in heat stressed birds with relatively slower growth rate and longer peripheral GH half life, seems in disagreement with this theory. GH produced by recombinant DNA technology is used in the treatment of growth disorders in children and may soon be applied in agriculture to increase efficiency of milk production in dairy herds. Its use in the production of lean meat is a possibility because chronic treatment of pigs with porcine GH increases muscle growth at the expense of adipose tissue (Etherton, 1989), although commercial application of this may rely on transgenic biology.

A fundamental problem underlying studies using exogenous cGH is that the role of endogenous cGH in the regulation of growth is not fully understood because lines of chickens genetically selected for rapid growth rate have lower serum cGH concentrations than slow growing lines (Burke and Marks, 1982; Goddard *et al.*,

1988). This result, again, challenges the existing theory that whether GH in heat stressed birds has the same well-defined functions including a central role in the control of growth and development as those in thermoneutral temperature.

In conclusion then, 1) Chronic heat stress reduces plasma concentrations of both T4 and T3 by mechanisms not involving the observed decreases in food intake; 2) Heat stress inhibits the stimulation of 5'-monodeiodination by GH despite an apparent enhancement of the GH response to TRH or exogenously administered GH; 3) Heat stress may increase the half life of circulating GH; 4) Changes in the control of thyroid hormone metabolism during chronic exposure to high environmental temperatures may, at least in part, account for the reduction in growth rate observed under these conditions.

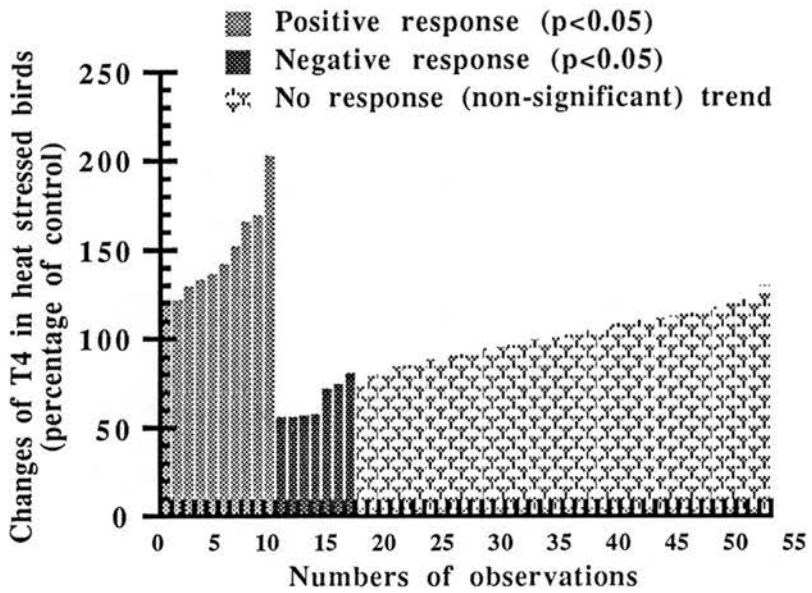
Chapter Four

The effects of a range of dry bulb temperatures upon conversion of T4 into T3 *in vivo* response to TRH in the domestic fowl

4.1. Introduction

Exposure to chronic high ambient temperature is known to influence endocrine systems in chickens. Exposure to high ambient temperature is known to result in changes in` the levels of T4 and T3. However, the existing literature on the effects of heat stress on thyroid hormones is very confusing. In the domestic fowl, an exposure to high ambient temperature may result in increased, decreased or unchanged plasma concentration of thyroxine (T4) and triiodothyronine (T3) (Figure 28). Figure 28 is based upon observations obtained from 15 papers described in Section 1.7.6.1 (Figures 8 and 9 in page 82-84).

Although it is well known that plasma T3 and GH concentrations decline as birds grow older, the same results obtained from the previous pair feeding studies using birds of different ages suggest that the conflicting results relating to the levels of T4 and T3 in heat stressed chickens may be not due to age. These conflicting results may be associated with the larger variability of performance in birds above 32 °C discussed earlier in Section 1.8 (Page 93-96). It is therefore possible that different dry bulb temperatures are a cause of the confusion in reported changes in the levels of T4 and T3 in heat stressed chickens. Variability in performance of birds above 32 °C might also be a consequence of the interaction of other factors (e.g. humidity, nutritional status, diet composition, stocking density, activity, acclimation, strain, sex, feather quality, or air movement), which may cause these conflicting results of the



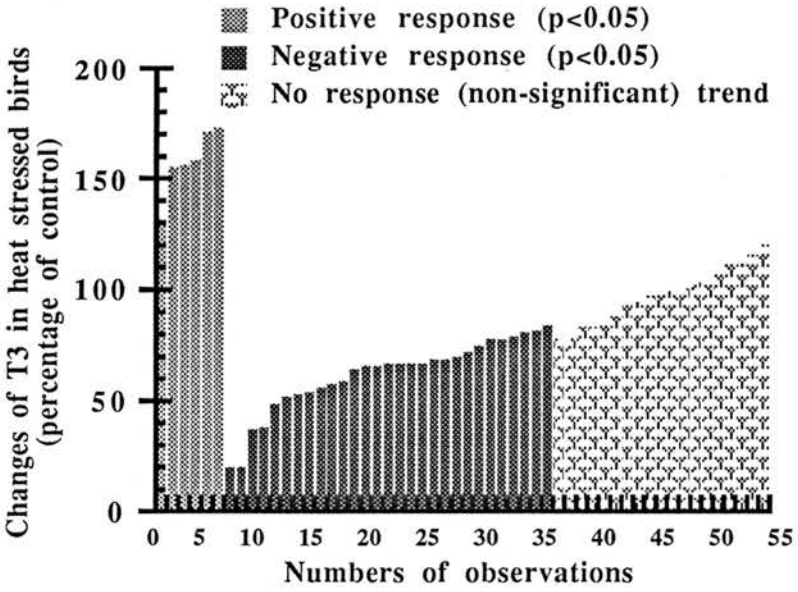


Figure 28. The conflicting results on the changes of thyroid hormones (percentage of control) in heat stressed birds obtained from 15 papers

All increased T3 concentration can be found only in acutely heat stressed birds. If we only consider chronic heat stress, no increased T3 concentration can be found. In other word, there is no conflicting result on the changes of T3 in chronically heat stressed birds.

thyroid function response to high ambient temperatures. These factors should, therefore, be considered and controlled in future experimental designs to minimise the risk of spurious results.

In this section the responses of the hypothalamus, the anterior pituitary gland, the thyroid gland and the 5'-monodeiodination to elevated exogenously administered thyrotrophin releasing hormone (TRH) *in vivo* have been examined in chickens exposed to a range of dry bulb temperatures. To our knowledge, the influence of the range of constant temperature of 21 to 35 °C on thyroid hormones and their response to TRH has not yet been studied. The aim of the experiment described herein was to determine the specific influence of the range of constant temperature of 25 to 35 °C with 35-45 % RH on growth rate and thyroid hormone mechanisms and their responses to subcutaneous TRH injections (10 μg/kg body weight) of broiler chickens from 3 to 6 weeks of age.

4.2. Experimental procedure

The data presented in this chapter were obtained from an experiment in which performance of chickens kept at high environmental temperatures (25 °C 45% RH, 29 °C 35% RH, 32 °C 35% RH and 35 °C 35% RH), or thermoneutral environmental temperature (21 °C 45% RH) were compared. Chickens, diets, housing, and measurements have been described in chapter 2. The experimental details were as follows. There were four trials in this experiment.

Commercial female broiler chickens (Ross Poultry (GB.) Ltd) between age 3 to 6 weeks were used in the experiment. The chickens were transported from chick brooder house (27 ± 1 °C) to one climate chamber at 21 ± 1 °C with 45 % RH and maintained on 23 hr light/day at 20 days of age. Four days after (at age of 24 days), they were randomly assigned to three groups of five each in the first, second and third

trials, and to three groups of six in the fourth trial. The grouping of birds into climate rooms in these four trials is described on Figure 29. Then, they were maintained on 23 hr light/day until 6-week of age. Food and water were provided *ad libitum*. All groups were fed a commercial pelleted broiler starter diet with 3000 kcal (12,552 kj) metabolisable energy and 230 g of crude protein /kg of diet. The birds' body temperatures were measured every two days using an electronic rectal probe. The body weights were measured every two days as well. Food intakes were measured daily and growth rate and feed conversion ratio were calculated. The data for growth performance was standardised and expressed as percentage in order to make the data comparable with other experiments using birds with different ages. Temperature and relative humidity in each room were controlled by the addition of conditioned air and ventilation. The actual temperature varied by no more than 1 °C around the set point. The apparent equivalent temperatures in each room were also calculated according to the mean values of the ambient temperature and relative humidity using the equation described in Section 2.5.2 (Page 118).

At the end of the experimental period, heparinised blood (2 ml) samples were obtained at 11:00 am from all birds by venipuncture before they received a subcutaneous injection of TRH (10 μg/kg body weight) in order to test the function of the hypothalamo-pituitary-thyroid axis and of stimulation of peripheral 5'-monodeiodinase activity in conversion of T4 to T3 in the chicken liver by GH, which elevates the circulating concentration of T3 (Mitchell *et al.*, 1985). Then, four further samples were taken at 40 minutes intervals post-injection. The blood plasma was prepared by centrifugation at 1500 g for 10 minutes and stored at -20 °C prior to assay for T4 and T3. Plasma concentrations of T4 and T3 were measured by radioimmunoassay (RIA) using commercially available kits (see Section 2.3.1, Page 114 for details).

Figure 29.

The method of grouping of birds into 3 climate rooms in four trials

Trial	Age (day)		Treatment		
			1	2	3
1	24-41	n=5	21 ± 1 °C 45 ± 5% RH	29 ± 0.2 °C 35 ± 5% RH	35 ± 0.2 °C 35 ± 5 % RH
2	24-41	n=5	21 ± 1 °C 45 ± 5% RH	29 ± 0.2 °C 35 ± 5% RH	35 ± 0.2 °C 35 ± 5 % RH
3	24-41	n=5	21 ± 1 °C 45 ± 5% RH	25 ± 0.2 °C 45 ± 5% RH	32 ± 0.2 °C 35 ± 5 % RH
4	24-41	n=6	21 ± 1 °C 45 ± 5% RH	24 ± 0.2 °C 45 ± 5% RH	29 ± 0.2 °C 35 ± 5 % RH

4.3. Results

The effects of a range of temperatures (21-35 °C, 35-45 % RH) representing the chronic moderate (29 °C 35% RH) and severe (32 °C 35% RH and 35 °C 35% RH) heat stress upon the depression of growth and a changed interaction of the body temperatures, the concentrations of T4 and T3 levels in the plasma of the chickens and their subsequent response to a subcutaneous injection of TRH are summarised in Figures 30-35.

Three weeks of heat stress caused a significant reduction in the plasma basal T4 and peak of T4 concentrations in chronic severe heat stressed broiler chickens ($P<0.001$ Figures 30 and 32a) but not in the chronic moderate heat stressed broiler chickens. In the control (21 °C 45% RH and 25 °C 45% RH) and the moderate heat stress group at 29 °C (with 35% RH), 10 μg TRH/kg body weight were effective in significantly ($P<0.01$) increasing plasma levels of T4 and T3 post-injection, while severe heat stress (32 or 35 °C) caused a significantly lower plasma peaks of T4 and T3 concentrations (Figures 30, 31 and 32). The inhibition of thyroid hormones and inhibition of their response to TRH after chronic severe heat stress in broiler chickens at or above 32 °C were associated with increases of body temperature above normal level (Figure 33), which are affected proportionally by dry bulb temperature (Figure 34).

Growth rate was affected proportionally by elevated dry bulb temperature *per se* (Figure 35). Whilst food conversion ratio was affected by heat stress although the effect was not affected proportionally by elevated dry bulb temperature. Feed conversion efficiency tend to be better (89-94% of feed conversion ratio - Figure 35) at moderate heat stress (29 °C / 35 %RH).

Figure 30. The effects of a range of dry bulb temperatures (relative humidity around 35-45 %RH) upon plasma T4 responses to the injection of TRH in individual trials. Values are expressed as means ± SE.

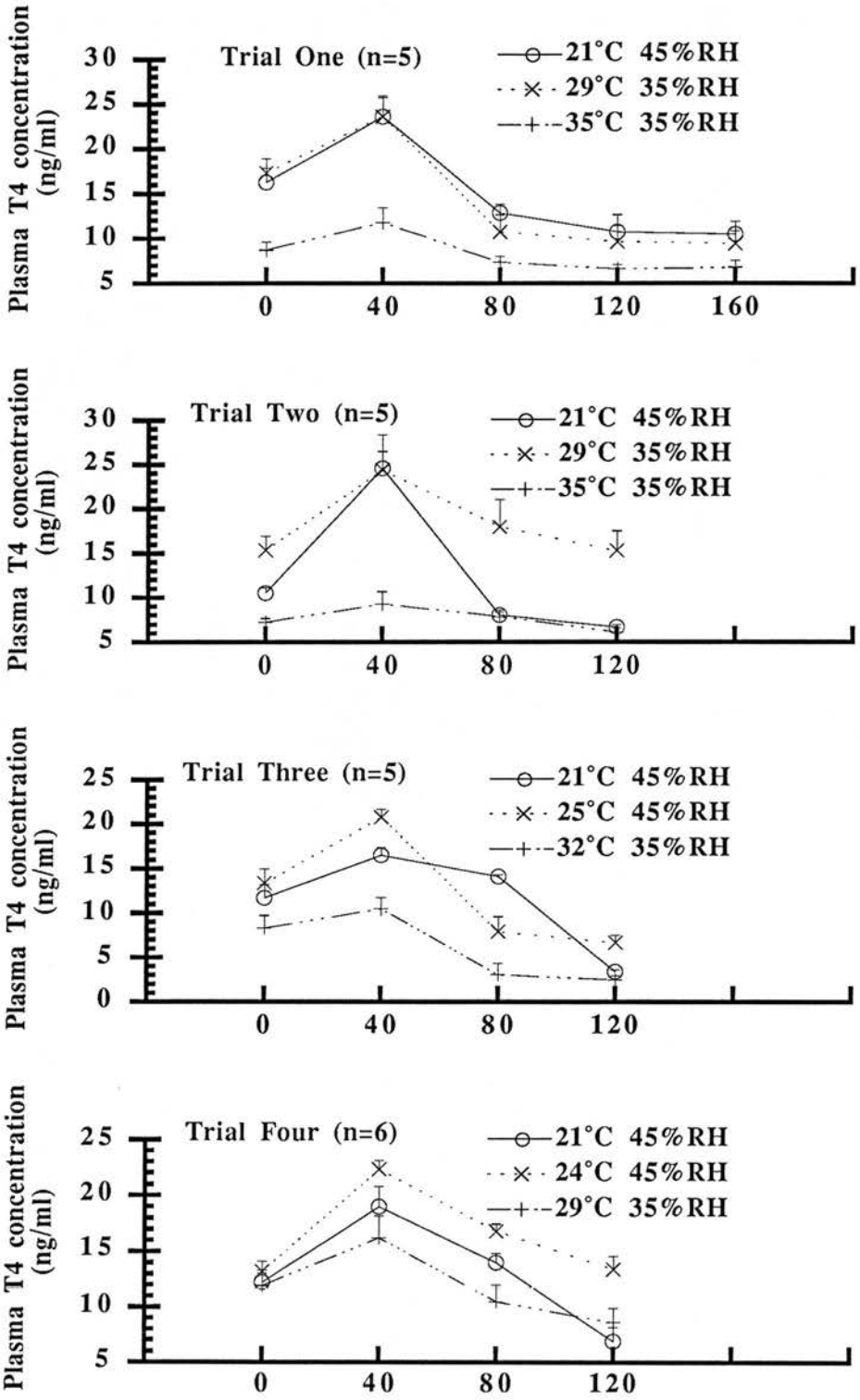


Figure 31. The effects of a range of dry bulb temperatures (relative humidity around 35-45 %RH) upon plasma T3 responses to the injection of TRH in individual trials. Values are expressed as means ± SE.

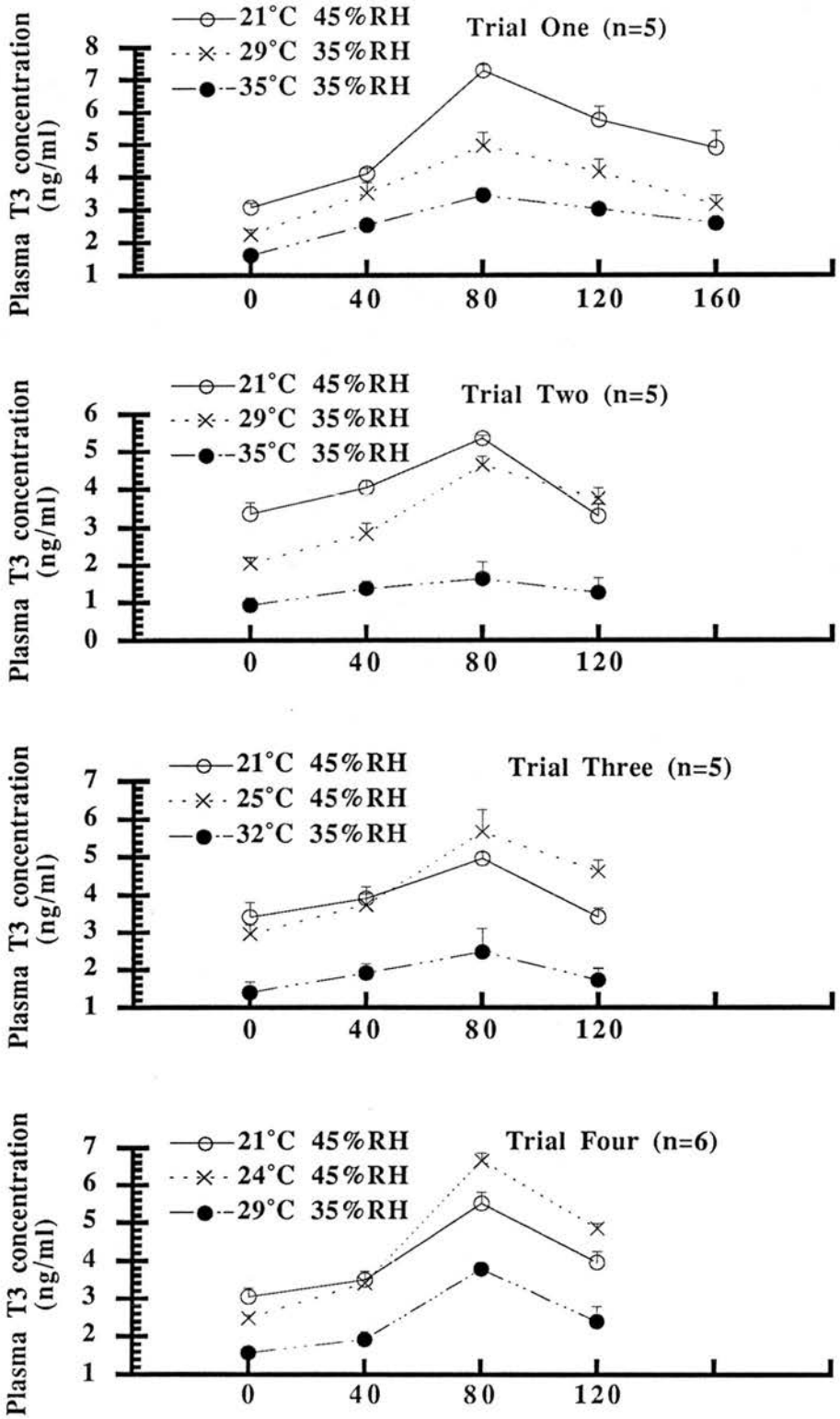


Figure 32. The effects of a range of dry bulb temperatures upon plasma T4 and T3 responses to the injection of TRH. Values are pooled from four trials and expressed as means ± SE.

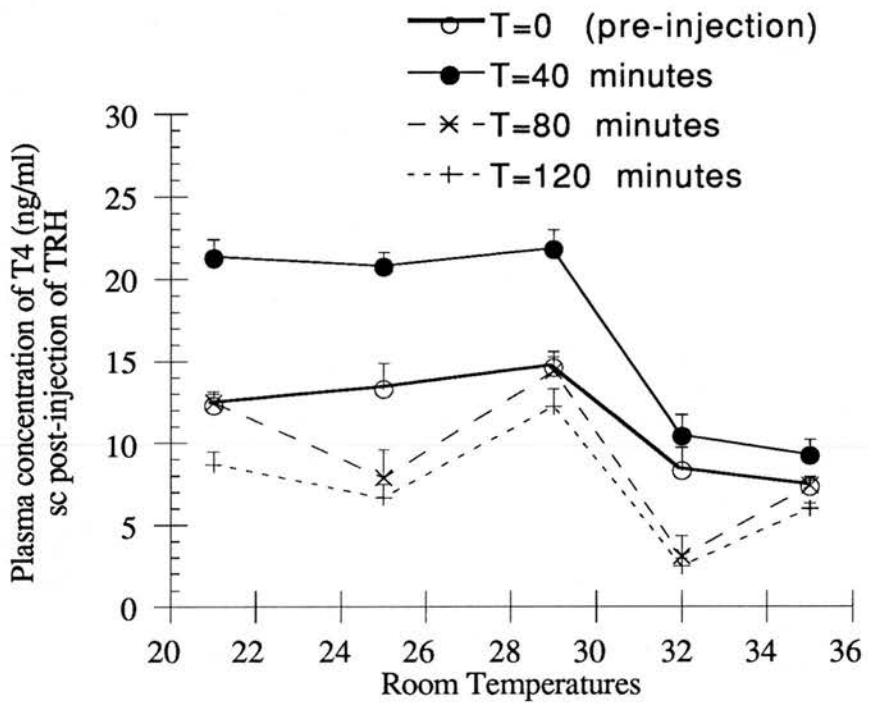


Figure 32a. Plasma T4 responses to the injection of TRH.

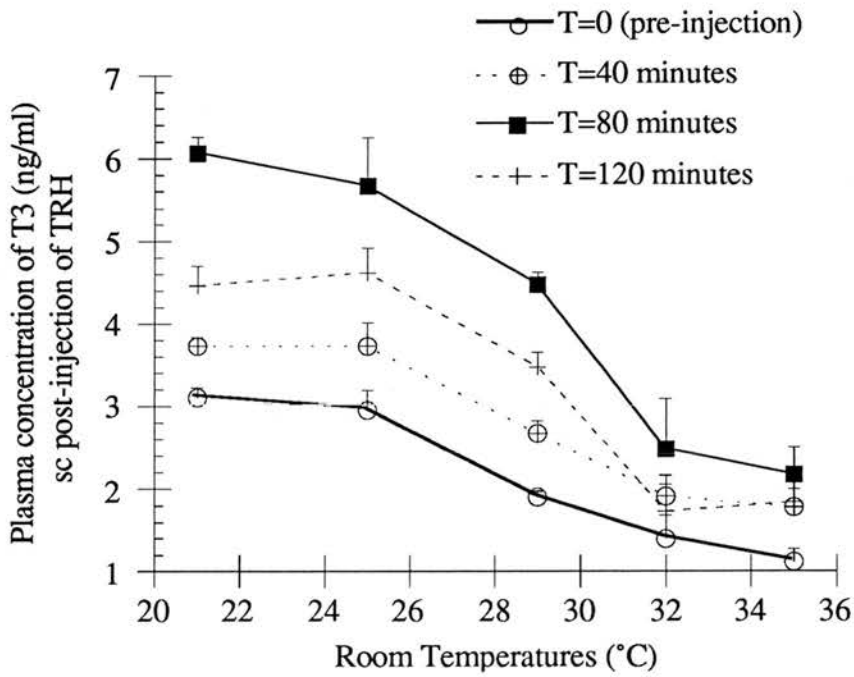


Figure 32b. Plasma T3 responses to the injection of TRH.

Figure 33. Effects of a range of dry bulb temperatures upon growth performances from 24 to 41 d and body temperatures in female broilers. Values are pooled from four trials and expressed as means ± SE.

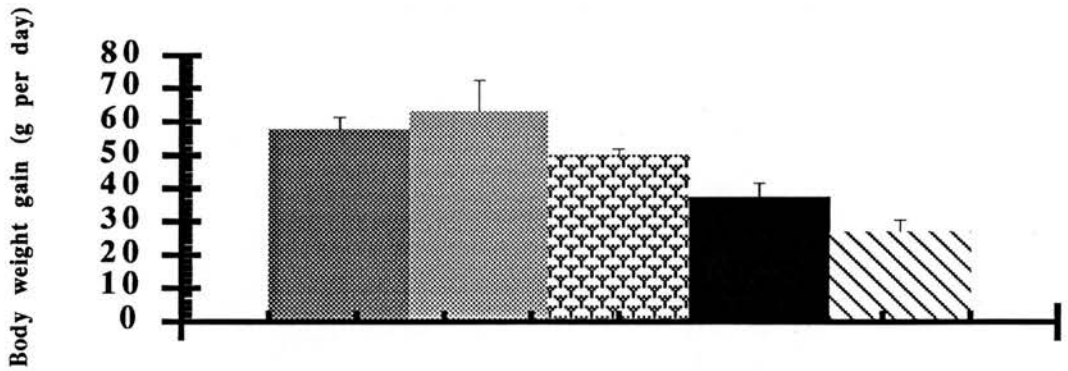


Figure 33a.

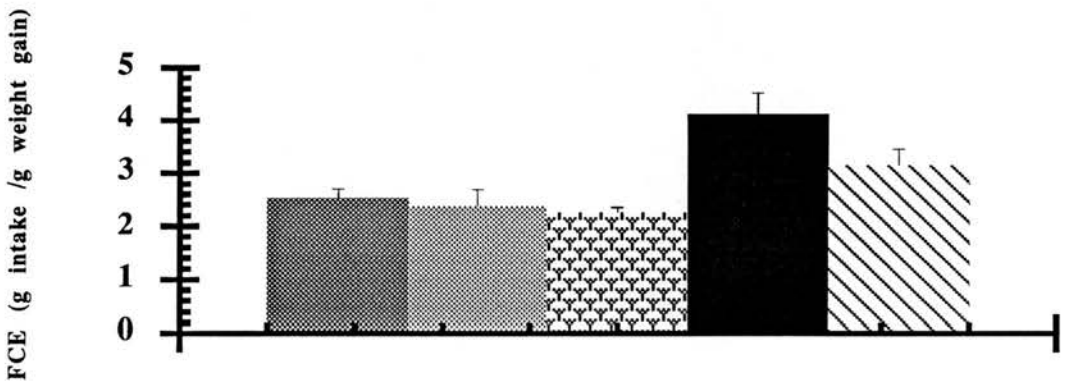


Figure 33b.

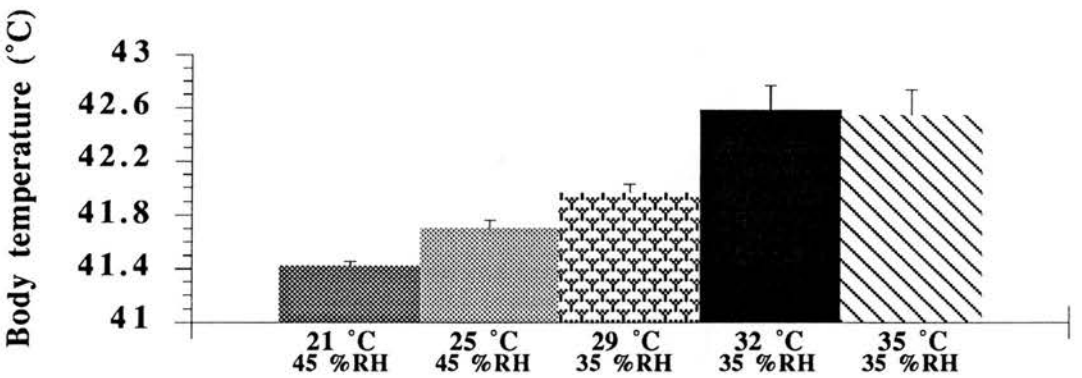


Figure 33c.

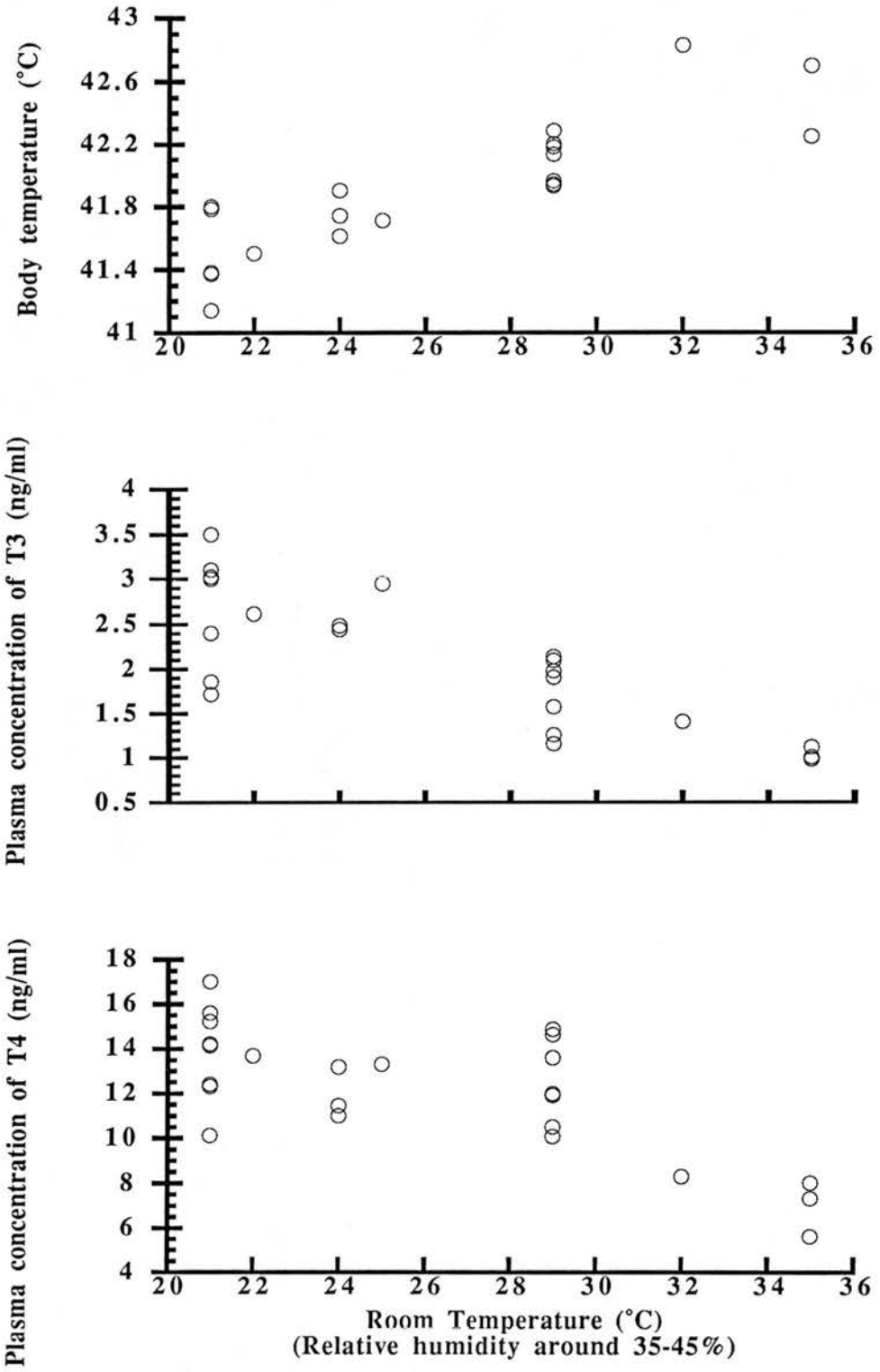


Figure 34. The influence of room temperatures upon changes of body temperatures (Tb) and hormonal concentrations. Values are presenting means obtained from all trials.

The regression equations are:

Tb (°C) = 39.6 + 0.0883 RoomTemperature (°C);
T3 (ng/ml) = 5.46 - 0.133 RoomTemperature (°C);
T4 (ng/ml) = 24.9 - 0.530 RoomTemperature (°C).

Figure 35. Comparison of (1) percentage of daily food intake (%), (2) percentage of daily weight gain (% - BWG), (3) percentage of feed conversion ratio (% - FCR) of broiler chickens at 21, 25, 29, 32 and 35 °C.

Measurement	Thermoneutral	Moderate heat stress		Severe heat stress	
	21 °C 45 %RH	25 °C 45 %RH	29 °C 35 %RH	32 °C 35 %	35 °C 35 %RH
Food Intake	100 a (149.72±12.592)	104 a (155.18±22.338)	71 c (107.033.9087)	87 b (129.72±12.782)	57 d (85.334.157)
BWG	100 a (57.584±3.7505)	110 a (63.167±9.4238)	87 b (49.9462.0513)	65 c (37.5±4.1105)	47 d (26.9333.6912)
FCR	100 b (2.527±0.1699)	94 ab (2.365±0.3225)	89 a (2.261±0.09203)	163 d (4.108±0.4128)	124 c (3.1432±0.30475)

Percentage of daily values are presented as the percentage of control birds at 21 °C for birds at 21, 25, 29, 32 and 35 °C.

Values within line with different letters are significantly different at $P<0.05$ level.

A higher value for feed conversion ratio is an index of poor feed conversion efficiency.

4.4. Discussion

The results indicated that plasma T4 concentration in chicken was affected by heat stress although the effect was not affected proportionally by elevated dry bulb temperature. Plasma T4 concentrations tend to be increased by 8% (Figure 32) at moderate heat stress (at 29 °C / 35 %RH). Plasma T4 concentration in chicken expose to above 32 °C was affected proportionally by elevated dry bulb temperature (Figure 34).

The finding provides evidence to explain the cause of the conflicting results relating to T4 and T3 responses in heat stressed chickens. The change in patterns of the plasma T4 concentration in chicken seems associated with food conversion ratio and the larger variability of performance in birds above 32 °C discussed earlier in Section 1.8 (Page 93-96).

It is concluded that chicken may be able to divide heat stress into a moderate one, which may only inhibit the capability of GH in stimulating hepatic 5'-monodeiodinating activity, and a severe one, which may cause TRH to become non-functional for hypophyseal stimulation of TSH and a lack of TSH-response to negative T3 feedback, since plasma T3 concentration in chicken was affected proportionally by elevated dry bulb temperature *per se* (Figures 32 and 34), and the responses of both plasma T3 and T4 concentrations to elevated exogenously administered thyrotrophin releasing hormone (TRH) have also been inhibited in chickens exposure to 32 °C or above 32 °C dry bulb temperatures.

In the current stage of knowledge it is difficult to explain the relative unresponsiveness of T4 and T3 to TRH in chickens kept at 32 and 35 °C although the higher responsiveness of GH to TRH in chickens kept at or above 29 °C. However, it has been shown previously that the peripheral production of T3 may depend on a

functional TRH-GH axis, since both TRH and GH are capable of stimulating hepatic 5'-monodeiodinating activity (Kühn *et al.*, 1986). It is therefore possible that during severe heat stress TRH becomes non-functional for hypophyseal stimulation of TSH but not that of GH and a lack of TSH-response could explain the absence of T4 increase while a lack of GH receptors to higher GH response could be responsible for the unresponsiveness of T3. The hypothesis could also explain the resistance of pre-conditioned chickens to severe heat stress increased survival time, and higher T3 with decreased survival time in non-conditioned chickens to acute severe hot condition (Fox, 1980) since an increased survival time in either radiothyroidectomized or thiouracil-treated chickens (Fox, 1980; Bobek *et al.*, 1983; Bowen and Washburn, 1985; Bowen *et al.*, 1984), broilers (May, 1982) or quails (Bowen and Washburn, 1985) exposed to 42 or 50 °C for up to 4 hours and a decreased survival time under similar conditions in birds injected with T3 or T4. The resistance of pre-conditioned chickens to severe heat stress increased survival time might be caused by unresponsiveness of T4 and T3 to TRH in chickens kept at severe hot condition. Whilst the higher T3 in non-conditioned chickens (Fox, 1980) and quails (Bobek *et al.*, 1980) to acute severe hot condition might be caused by the responsiveness of T4 and T3 to TRH in birds kept at thermoneutral or moderate heat stress condition.

Thus, in conclusion, the lowest rate of growth in chronic heat stressed chickens is accompanied by a decrease in T3 concentration followed by a slight increase or unchanged in plasma T4 at moderate heat stress condition or a decrease at severe heat stress condition in plasma T4. These effects may be related to the extent of hyperthermia induced by a specific heat load but are not the consequence of concomitant decreases in food intake. The severely depressed growth performance associated with the inhibition of thyroid hormones and their response to TRH after chronic severe heat stress in broiler chickens did not occur in the chronic moderate heat stressed broiler chickens. The better feed conversion efficiency (lower feed

conversion ratio) in moderate heat stressed broiler chickens but worse feed conversion efficiency (higher feed conversion ratio) in severe heat stressed broiler chickens association with plasma concentrations of basal and peak of T4 in the chronic heat stressed broiler chickens were found. When assessing the effects of "hot" climates or heat stress upon endocrine function and growth in chickens, the environments should be characterised in terms of their potential influence upon thermal exchange and thermoregulation and chronic heat stress conditions reduces circulating T3 possibly by a reduction in hepatic 5'-monodeiodination.

In conclusion then, 1) Chickens might be able to divide high ambient temperatures into moderate and severe heat stress conditions. Heat stress caused a significant reduction in plasma T4 concentration in chronic severe heat stressed broiler chickens but not in the chronic moderate heat stressed broiler chickens. 2) The failure of TRH to induce T4 release in chronic severe heat stressed birds may be due to an impairment in pituitary (TSH) function. 3) Differences in the control of thyroid hormone metabolism during chronic exposure to different high environmental temperatures may, at least in part, account for the reduction in growth rate observed under these conditions.

Chapter Five

The effects of differing heat loads upon plasma concentrations of thyroid hormones and growth hormone in the domestic fowl

5.1. The effects of differing heat loads upon body temperatures, plasma baseline concentrations of thyroid hormones and growth hormone are different

5.1.1. Introduction

Recent observations suggest that chickens might be able to divide high ambient temperatures into moderate and severe heat stress conditions. The influence of a range of dry bulb temperatures of 21 to 35 °C with low humidity on plasma T4 and T3 or their response to exogenously administered TRH *in vivo* have demonstrated that the T4 response was greatly decreased by severe heat stress but was unaltered by the moderate treatment despite elevated absolute concentrations. The different hormonal levels among birds exposed to different environmental temperatures suggest that the precise control of environmental temperature for good research is important.

The confusion in responses in the levels of T4 and T3 in heat stressed chickens may also be affected by different humidity in the humid production areas if chickens are able to divide high ambient temperatures into moderate and severe heat stress conditions, because humidity of the ambient air could be a critical factor in heat dissipation from the heat stressed chicken. Heat stress is usually described in relation to elevated dry bulb temperatures but water vapour density (VD) also has important effects upon heat exchange under such conditions as discussed in Section 1.6.3 (Page

54). The comparison of different heat loads by apparent equivalent temperatures (AET) at ambient temperature of 21, 22, 24, 25, 29, 32 and 35 °C with different humidities is shown in Figure 36. The apparent equivalent temperature is higher at 29 °C / 85%RH than at 35 °C / 35%RH (Figure 36). As environmental temperature increases, heat loss from the chicken's respiratory tract in the latent form becomes progressively more important (Ota *et al.*, 1953). Consequently, for ambient temperatures near 41 °C, the relative humidity of the ambient air should be a critical factor in heat loss from the chicken.

It was therefore considered appropriate to study the effects of different heat loads upon the endocrine responses of broiler chickens. Heat load was altered by controlling water vapour density at either 10.1 or 24.4 gm^{-3} at a single elevated dry bulb temperature (29 °C) corresponding to RH values of 35 and 85%. These conditions were defined as moderate and severe chronic heat stress respectively. Control birds were maintained at 21 °C, VD=8.3 gm^{-3} (RH 45%).

5.1.2. Experimental procedure

All experiments were performed on individually caged birds in climate chambers in which both temperature and relative humidity were accurately controlled (±0.2 °C; ±5% RH). Birds were exposed to each of the 3 thermal loads for 17 days from 24 to 41 days of age in the first and the second trials, and for 28 days from 30 to 58 days of age in the third trial. Control birds were maintained at 21 °C / 45% RH in the first and the second trials, and 21 °C / 60% RH in the third trial. Moderate chronic heat stressed birds (MH) were maintained at 29 °C / 35% RH in the first and second trials, and at 29 °C / 50% RH in the third trial. Severe chronic heat stressed birds (HHS) were maintained at 29 °C and 85% RH in all trials. Plasma samples were obtained at 11:00 am from 6 birds in the first trial by venipuncture (brachial vein) in each treatment at intervals throughout this period. Whilst plasma samples were

Figure 36. Comparison of different heat loads by apparent equivalent temperatures (AET) at ambient temperature of 21, 22, 24, 25, 29, 32 and 35 °C with ambient different humidities.

Ambient temperature		Ambient humidity		Apparent equivalent temperatures
°C	°F	RH (%)	VD (gm^{-3})	AET (°C)
21	69.8	45	8.25135	39.24543
21	69.8	60	11.0018	45.32724
22	71.6	45	8.74248	41.37844
24	75.2	45	9.80093	45.82968
25	77.0	45	10.37021	48.15307
29	84.2	35	10.06550	51.68700
29	84.2	50	14.37929	61.40999
29	84.2	85	24.44480	84.09699
32	89.6	35	11.83151	58.85505
35	95.0	35	13.85597	66.66859
35	95.0	45	17.81482	75.71675

obtained at 11:00 am from 6 birds in the third trial, and from 5 birds in the second trial by venipuncture (brachial vein) in each treatment at end of this period. In all animals of 30 birds in each treatment, food intakes and body weight gain were determined daily in addition to monitoring deep body temperature in the first trial. Plasma concentrations of thyroxine (T4) and tri-iodothyronine (T3) were determined by radioimmunoassay. Plasma concentrations of GH were measured by enzyme-linked immunosorbent assay (ELISA) using the method of Houston *et al.* (1991) based on two monoclonal antibodies against cGH (Goddard *et al.*, 1987). The ELISA was highly specific for cGH and showed no cross-reactivity with other pituitary hormones (Houston *et al.*, 1991).

Heat load was altered by controlling water vapour density at either 10.1 or 24.4 gm^{-3} at a single elevated dry bulb temperature (29 °C) corresponding to RH values of 35 and 85%. These conditions were defined as moderate and severe chronic heat stress respectively. Control birds were maintained at 21 °C, VD=8.3 gm^{-3} (RH 45%). Water vapour density (VD) can be calculated by the following equation (Monteith, 1973).

$$VD\ (gm^{-3}) = \frac{0.622\ Pa\ e}{(p - e)}$$

where water vapour density (VD) and air vapour density (P_a) are expressed in the same unit of gm^{-3}; where water vapour pressure (e) and air vapour pressure (p) are expressed in the same unit of mbar. Water vapour density (VD) is often called the absolute humidity. When ambient temperature is 20 °C, P_a =1204 gm^{-3}; when ambient temperature is 25 °C, P_a =1183 gm^{-3} ; when ambient temperature is 30 °C, P_a =1.164 gm^{-3}. The apparent equivalent temperatures in each room were also calculated according to the mean values of the ambient temperature and relative humidity using

the equation described in Section 2.5.2 (page 118). The apparent equivalent temperature is higher at 29 °C / 85%RH than at 35 °C / 35%RH (Figure 36).

5.1.3. Results

The effects of different thermal loads upon body weight, body temperature, and the baseline plasma T3, T4 and GH concentrations in female broilers are summarised in figures 37-43.

As expected, the 17-day chronic severe and moderate heat stress treatments had pronounced effects on body weight and body temperature. Growth rates were depressed (Figure 37) and body temperatures elevated (Figure 38) to a greater degree by the higher heat load. The results indicated that when assessing the effects of "hot" climates or heat stress upon growth in chickens, the environments should be characterised in terms of their potential influence upon thermal exchange and thermoregulation and not simply by measurement of dry bulb temperature.

Heat stress markedly reduced plasma T3 concentration within 3 days of exposure ($p<0.05$) an effect which was greater at the higher VD ($p<0.001$ - Figures 39 and 42). It is apparent that both chronic severe and moderate heat stress reduce circulating T3 and greater effect at the higher VD ($p<0.001$ - Figures 39 and 42) suggests that an effect of chronic heat stress reducing circulating T3 may be proportional to heat load rather than temperature *per se*. Plasma T4 was reduced in broilers subject to chronic severe heat stress but plasma T4 was increased in broilers subject to chronic moderate heat stress ($p<0.01$) during the last week of exposure (Figure 40) compared to control birds, although there was no significant difference in circulating GH between the 2 groups at 29 °C in response to altered VD. The increased plasma T4 in moderate heat stressed broilers (29 °C / 50%RH) was, however, not constant when humidity increased (Figure 43). These results have demonstrated that

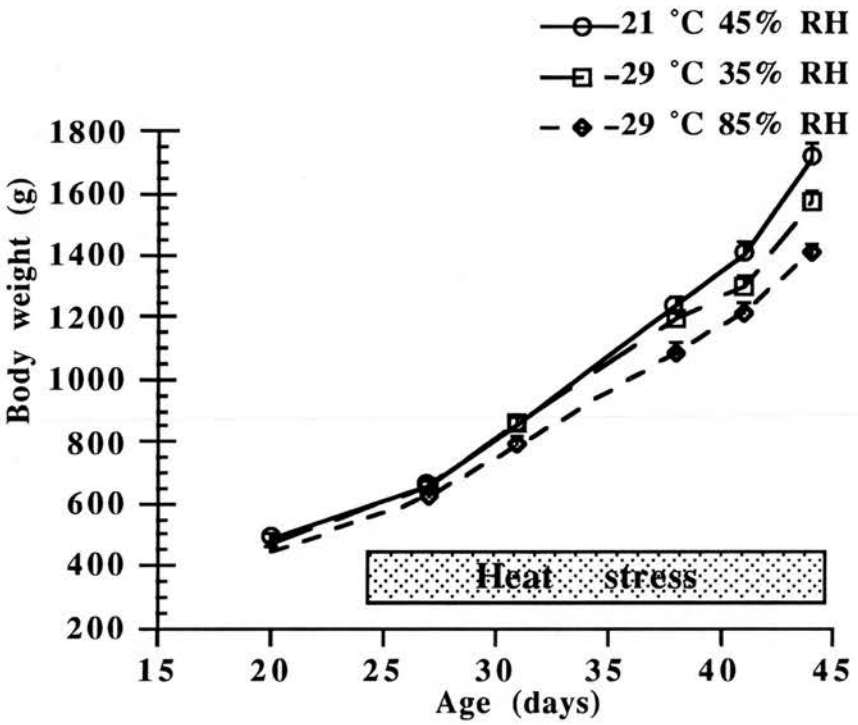


Figure 37. The effects of different thermal loads upon body weight in female broilers. Values are expressed as means ± SE for thirty female broiler chickens in Trial One.

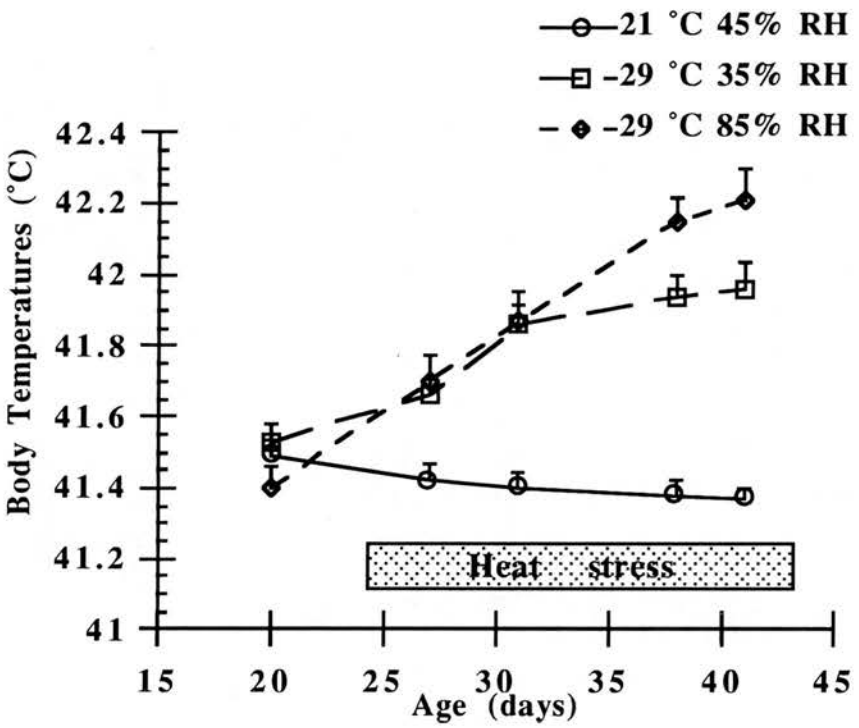


Figure 38. The effects of different thermal loads upon body temperature in female broilers. Values are expressed as means ± SE for thirty female broiler chickens in Trial One.

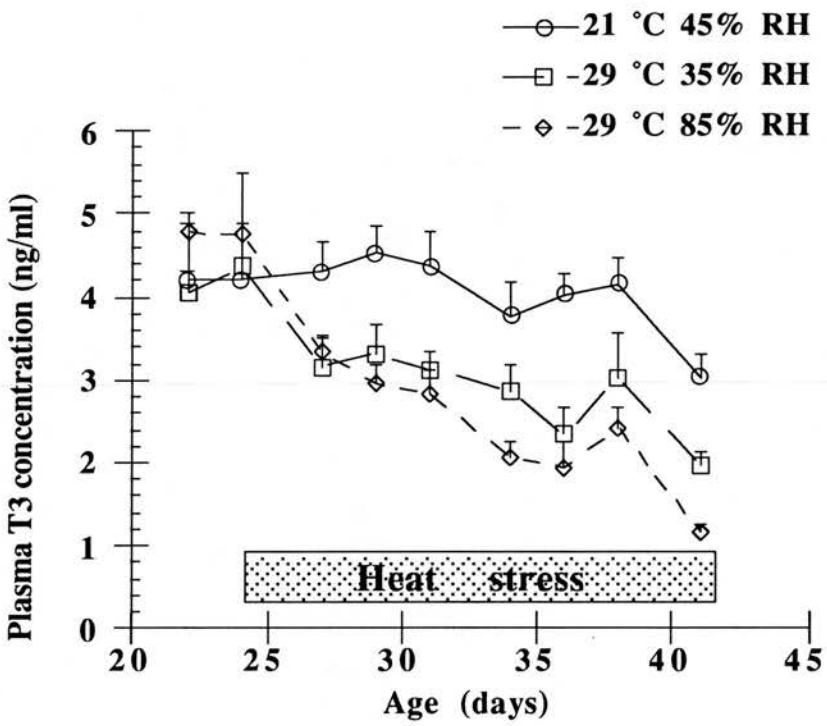


Figure 39. The effects of different thermal loads upon plasma T3 concentration in female broilers. Values are expressed as means ± SE for six female broiler chickens in Trial One.

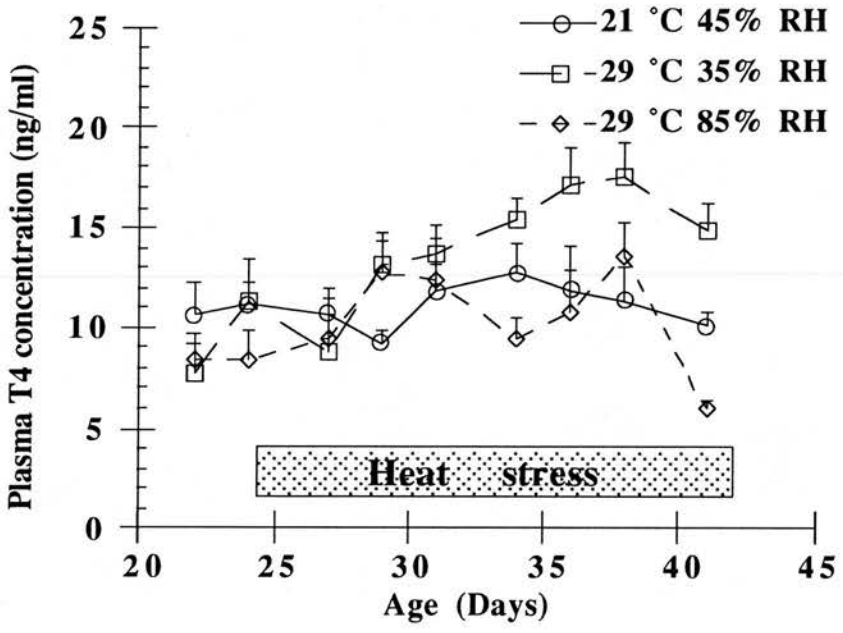


Figure 40. The effects of different thermal loads upon plasma T4 concentration in female broilers. Values are expressed as means ± SE for six female broiler chickens in Trial One.

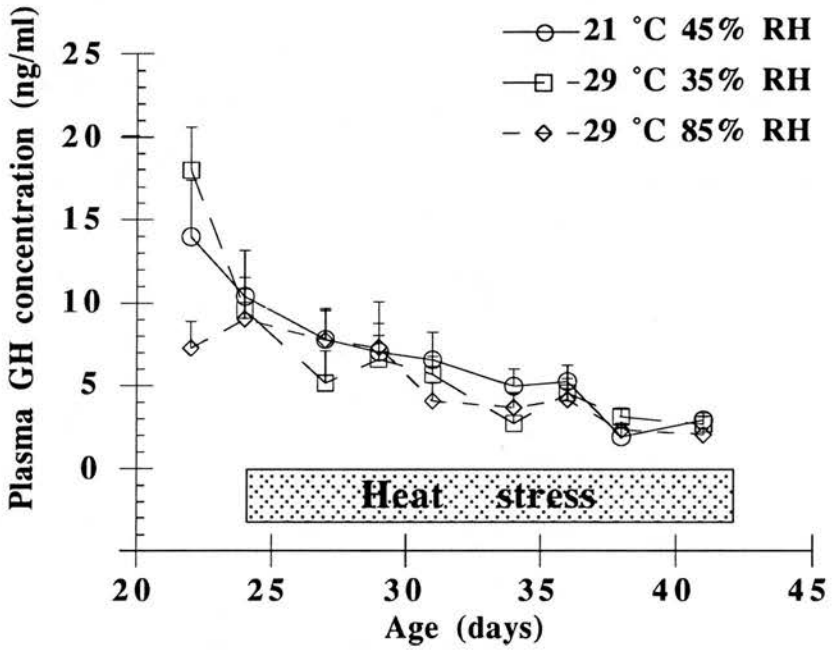


Figure 41. Comparison of different thermal loads upon the baseline plasma GH concentration (ng/ml) in female broilers. Values are expressed as means ± SE for six female broiler chickens in Trial One.

Figure 42. The effects of different thermal loads upon plasma T3 concentration in the second and the third trials. Values are expressed as means ± SE.

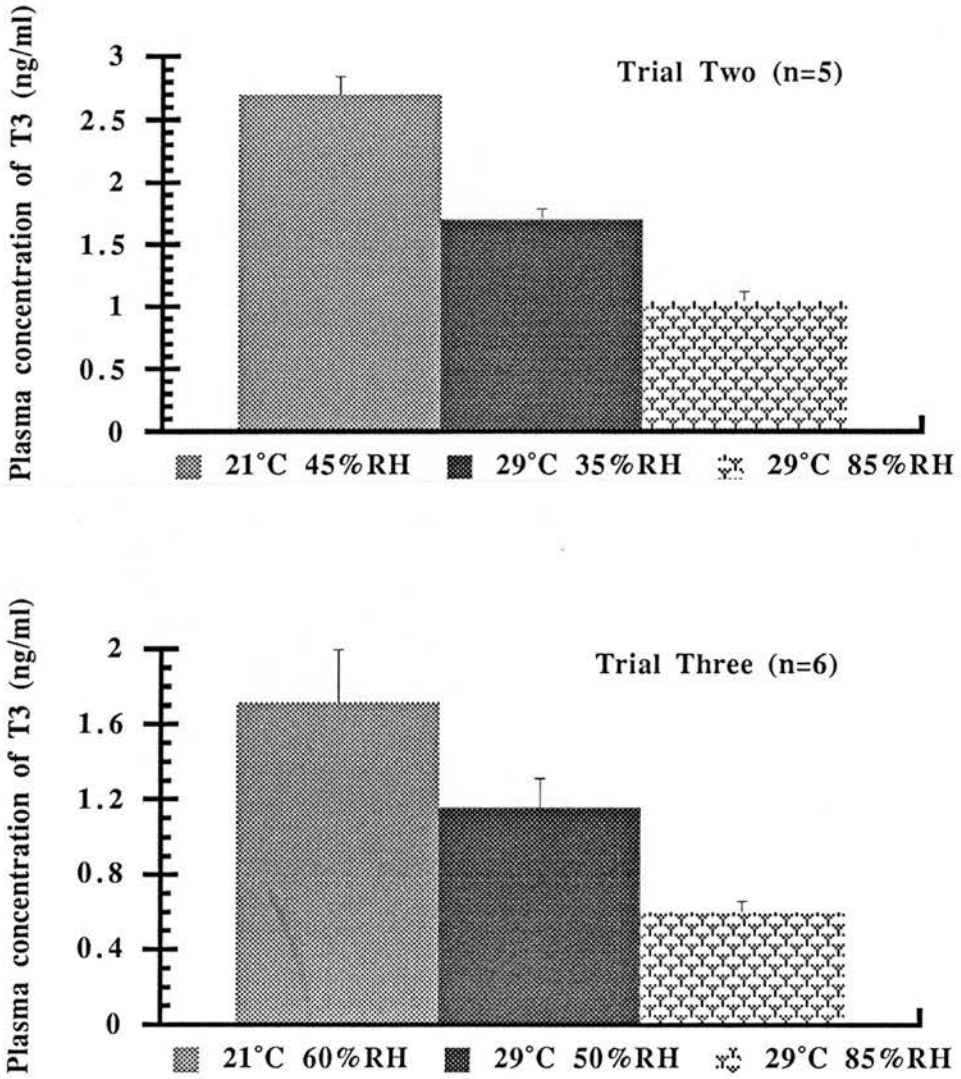
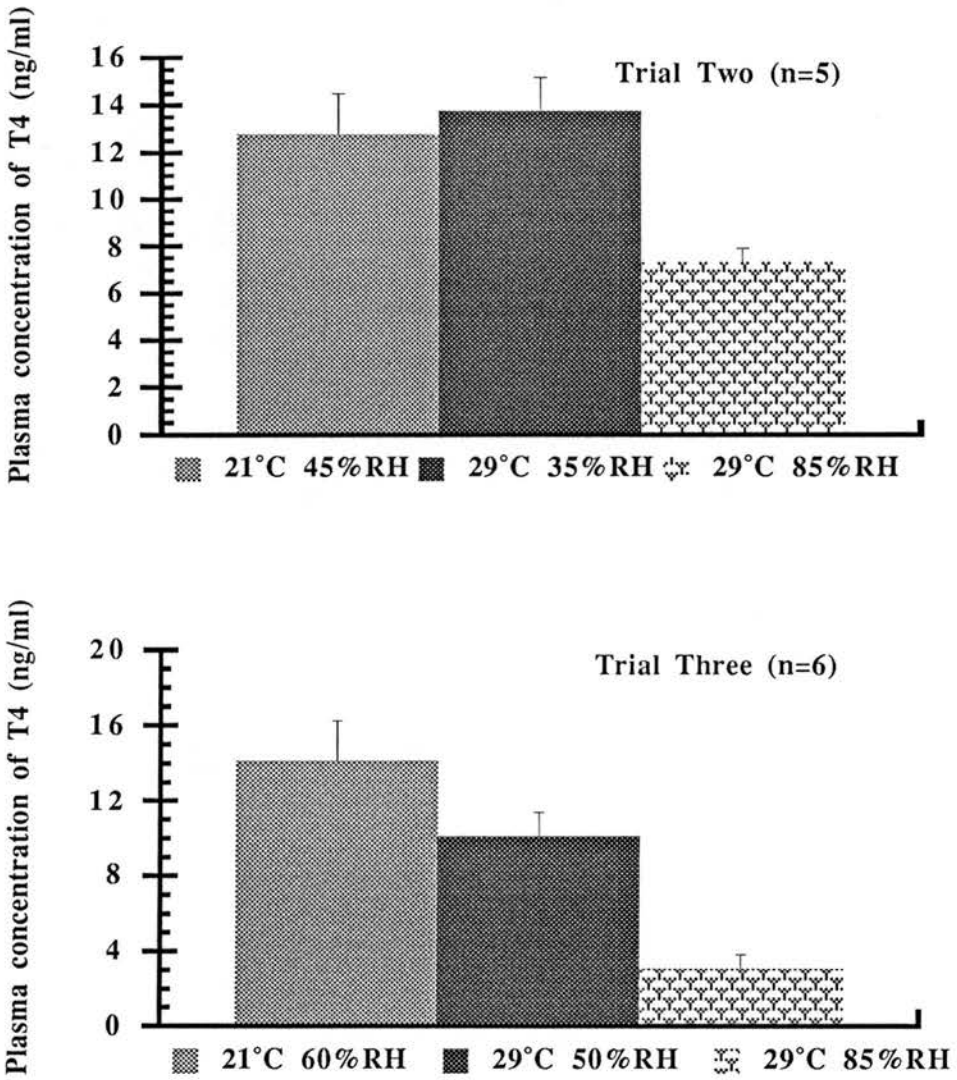


Figure 43. The effects of different thermal loads upon plasma T4 concentration in the second and the third trials. Values are expressed as means ± SE.



this phenomenon may be mediated by a reduction in hepatic 5'-monodeiodination and may involve changes in the function and interaction of the thyrotrophic and somatotrophic axes.

GH concentrations decreased in all groups over the experimental period but with no significant effect of heat load (Figure 41). This result confirmed the finding of GH higher during the "rapid growth phase" of broiler chickens. Within lines, GH is often positively correlated with relative gain (growth rate) (Burke and Marks, 1982), particularly when birds are evaluated temporally, such that pituitary GH synthesis (Hoshino and Yamamoto, 1977) and circulating GH decrease as growth rate declines with post-hatch age for chickens (Vasilatos-Younken and Zarkower, 1987) and turkeys (Proudman and Wentworth, 1980; Vasilatos-Younken *et al.,* 1988b). Thus, plasma GH is higher during the early period of most rapid growth and lower during slower, late growth. In contrast, hepatic GH binding increases with age, concurrent with decreasing plasma GH and growth rate (Leung *et al.*, 1984b; Leung *et al.,* 1987b; Vasilatos-Younken *et al.,* 1990).

Although both plasma T3 and GH concentrations are affected by age, the experiment shows the heat load rather than the age of birds influences the changes in thyroid hormone levels in heat stressed birds.

Heat stress markedly reduced plasma T3 concentration within 3 days of exposure and growth rates were depressed. These effects were greater at the higher VD. The apparent effects suggest that an effect of chronic heat stress reducing circulating T3 and growth rates may be proportional to heat load rather than temperature *per se*. The effect of T4 and particularly T3 on growth in birds is critically determined by the dose administered or endogenously present in the bird. While low-to-intermediate levels of T3 and T4 are required for growth, high doses of T3 and to a lesser extent of T4 will depress growth. It should be note that plasma T4 was reduced

in broilers subject to chronic severe heat stress but plasma T4 was increased in broilers subject to chronic moderate heat stress ($p<0.01$) during the last week of exposure (Figure 40) compared to control birds, although there was no significant difference in circulating GH between the 2 groups at 29 °C in response to altered VD.

These results indicate that the confusion relating to T4 and T3 responses in heat stressed chickens in the existing literature (see Figure 28 in Section 4.1, Page 140 for details) may be the result of different humidities in different studies, and the depressed growth rate in chickens exposed to heat stress may be a consequence of the different levels of T4 and T3. Heat stress is usually described in relation to elevated dry bulb temperatures but water vapour density (VD) also has important effects upon heat exchange under such conditions. As environmental temperature increases, heat loss from the chicken's respiratory tract in the latent form becomes progressively more important (Ota *et al.*, 1953).

5.2. The effects of differing heat loads upon conversion of T4 into T3 and plasma GH responses to TRH are different

5.2.1. Introduction

In this section the responses of the hypothalamus, the anterior pituitary gland, the thyroid gland and the 5'-monodeiodination to elevated exogenously administered thyrotrophin releasing hormone (TRH) have been examined in terms of the effects on plasma concentrations of thyroxine (T4), tri-iodothyronine (T3) and growth hormone (GH) in order to establish if changes in thyroid hormone economy in response to heat stress are caused by interactions of the thyrotrophic-somatrophic axis.

It has been well established that thyrotrophin releasing hormone (TRH) stimulates GH secretion in young chickens. The hypothalamic hormones which release GH or itself stimulate the peripheral conversion of T4 into T3 in the chick embryo (Kühn *et al.*, 1988a). Injections of mammalian somatotrophin stimulated embryonic conversion of T4 to triiodothyronine (T3) (Kühn *et al.*, 1986; Berghman *et al.*, 1989).

To our knowledge, the influence of environmental temperature with different humidities on T4, T3 and GH response to TRH is not yet known. However, GH responses of immature broilers to TRH stimulated 5'-monodeiodination (Scanes *et al.*, 1983). Since temperature changes thyroid hormone levels, a changed interaction of the thyrotrophic-somatrophic axis may result in an altered GH response to TRH in the anterior pituitary gland, TSH response to TRH in the anterior pituitary gland, T4 response to TSH in the thyroid gland, 5'-monodeiodination response to GH in the liver and TRH response to negative T3 feedback in the hypothalamus.

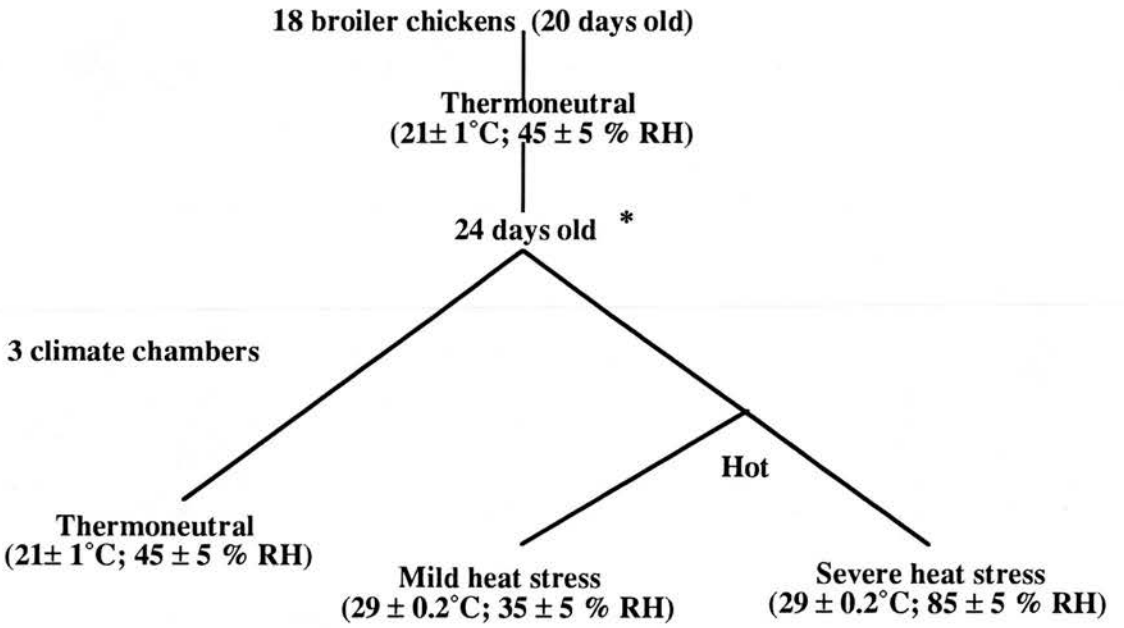
These experiments, similar to the previous one, was carried out to investigate the effects of different thermal loads at a single ambient temperature (29 °C) achieved by varying the humidity (35 or 85% RH), in order to test the function of the hypothalamo-pituitary-thyroid axis and the thyrotrophic-somatrophic axis and to examine the GH control of 5'-monodeiodination response to TRH in heat stressed broilers, (Mitchell, 1987b; Mitchell and Goddard, 1990). It was therefore considered appropriate to study plasma T4, T3 and GH responses to exogenously administered TRH during chronic heat stress growing broiler chickens between 3 and 6 weeks of age.

5.2.2. Experimental procedure

All experiments were performed on individually caged birds in climate chambers in which both temperature and relative humidity were accurately controlled (±0.2 °C; ±5% RH). Birds were exposed to each of the 3 thermal loads for 17 days from 24 to 41 days of age in the first trial (see Figure 44 for details). Control birds were maintained at 21 °C and 45% RH. Moderate chronic heat stressed birds (MH) were maintained at 29 °C and 35% RH. Severe chronic heat stressed birds (HHS) were maintained at 29 °C and 85% RH. Plasma samples were obtained at 11:00 am from 6 birds by venipuncture (brachial vein) in each treatment at end of this period. At the end of exposure further samples were taken immediately prior to and at 40 minute intervals following the subcutaneous administration of 10 µg TRH/kg body weight. In all animals of 30 birds in each treatment , food intakes and body weight gain were determined daily in addition to monitoring deep body temperature. Plasma concentrations of thyroxine (T4) and tri-iodothyronine (T3) were determined by radioimmunoassay. Plasma concentrations of GH were measured by enzyme-linked immunosorbent assay (ELISA) using the method of Houston *et al.* (1991) based on two monoclonal antibodies against cGH (Goddard *et al.*, 1987). The ELISA was highly specific for cGH and showed no cross-reactivity with other pituitary hormones (Houston *et al.*, 1991).

Birds were exposed to each of the 3 thermal loads for 24 days from 20 to 44 days of age in the second and the fourth trials, for 17 days from 24 to 41 days of age in the third trial. Control birds were maintained at 22 °C / 45% RH, 21 °C / 45% RH and 24 °C / 45% RH in the second, the third and the fourth trials, respectively. Moderate chronic heat stressed birds (MH) were maintained at 29 °C / 35% RH in all trials. Severe chronic heat stressed birds (HHS) were maintained at 29 °C and 85% RH in all trials. Plasma samples were obtained at 11:00 am from 5 birds in the second

Figure 44. Diagram of experimental procedure



Note:* All were kept in 1 climate chamber for adaptation at 21± 1°C; 45 ± 5 % RH until 24 days old.

and the third trials, and from 6 birds in the fourth trial by venipuncture (brachial vein) in each treatment at end of this period. At the end of exposure further samples were taken immediately prior to and at 40 minute intervals following the subcutaneous administration of 10 μg TRH/kg body weight. Plasma concentrations of thyroxine (T4) and tri-iodothyronine (T3) were determined by radioimmunoassay.

Heat load was altered by controlling water vapour density at either 10.1 or 24.4 gm^{-3} at a single elevated dry bulb temperature (29 °C) corresponding to RH values of 35 and 85% (see Section 5.1.2, page 148 for details).

5.2.3. Results

The effects of different thermal loads upon the interaction of plasma T3, T4 and GH concentrations representing the thyrotrophic-somatrophic axis response to TRH in female broilers are summarised in figures 45-50.

As expected, the 17-day chronic severe and moderate heat stress treatments had pronounced effects on food intake, body weight gain and food conversion ratio (Figure 45). These parameters were affected proportionally by elevated apparent equivalent temperatures (Figure 45).

The chronic severe and moderate heat stress treatments had pronounced effects on the thyrotrophic-somatrophic axis response to TRH. The T3 response to TRH was reduced by moderate thermal loads (29 °C/35% RH, $p< 0.01$) with the greatest inhibition in severe heat stress (29 °C/85% RH, $p<0.001$ - Figures 46 and 49). The T4 response was greatly decreased by severe heat stress but was unaltered by the moderate treatment despite elevated absolute concentrations in Trial One (Figures 47 and 50). These effects were accompanied by a 3 fold increase ($p<0.001$) in the TRH induced stimulation of GH secretion (Figure 48).

Figure 45. Comparison of (1) percentage of daily food intake (%), (2) percentage of daily weight gain (% - BWG), (3) percentage of feed conversion ratio (% - FCR) of broiler chickens at 21 °C, 29 °C with low and high relative humidity (RH).

Measurement	Thermoneutral	Moderate heat stress	Severe heat stress
AET* (°C)	39.2	51.7	84.1
Dry bulb temperature (°C)	21 °C	29 °C	29 °C
Relative humidity (RH)	45 %RH	35 %RH	85 %RH
Absolute humidity (gm^{-3})	8.3	10.1	24.4
Food Intake	100 a (124.94±5.6502)	83 b (104.19±2.7738)	70 c (87.68±2.73087)
BWG	100 a (55.403±1.7699)	85 b (47.099±1.60208)	76 c (42.335±1.4072)
FCR	100 a (2.456±0.1375)	95 ab (2.324±0.10561)	87 b (2.1325±0.10895)

* AET = apparent equivalent temperature.

Percentage of daily values are presented as the percentage of control birds at 21 °C for birds at 29 °C with low and high relative humidity (RH) (N=30).

Values within line with different letters are significantly different at $P<0.05$ level.

A higher value for feed conversion ratio is an index of poor feed conversion efficiency.

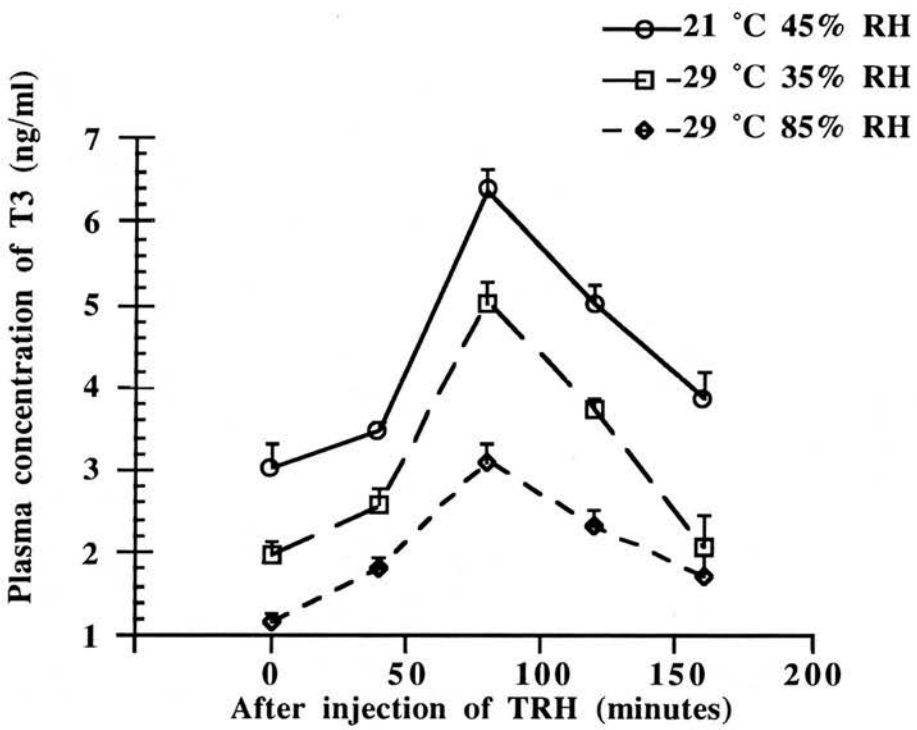


Figure 46. The effects of different thermal loads upon plasma T3 responses to the injection of TRH. Values are expressed as means ± SE for six female broiler chickens in Trial One.

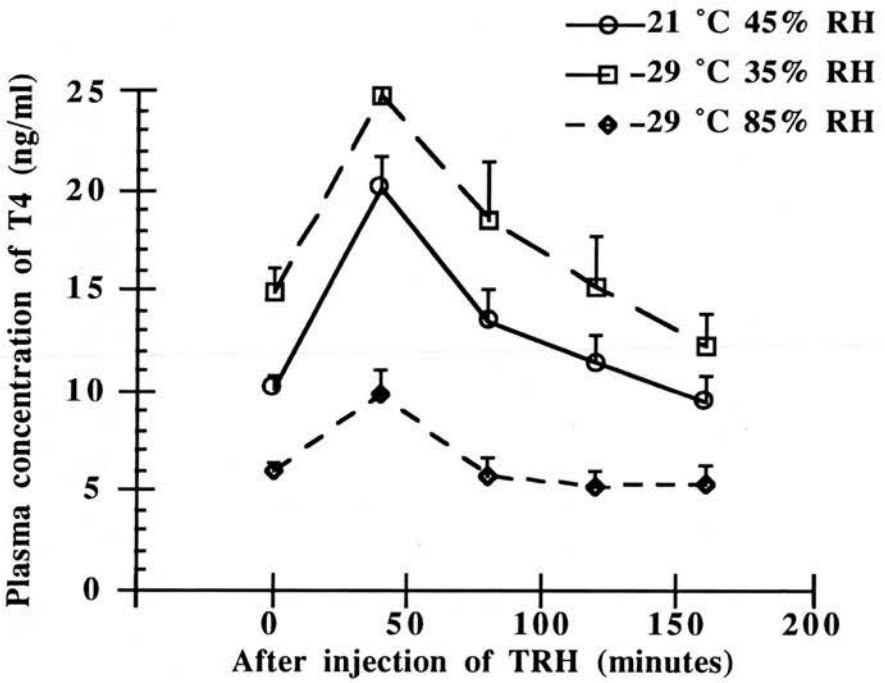


Figure 47. The effects of different thermal loads upon plasma T4 responses to the injection of TRH. Values are expressed as means ± SE for six female broiler chickens in Trial One.

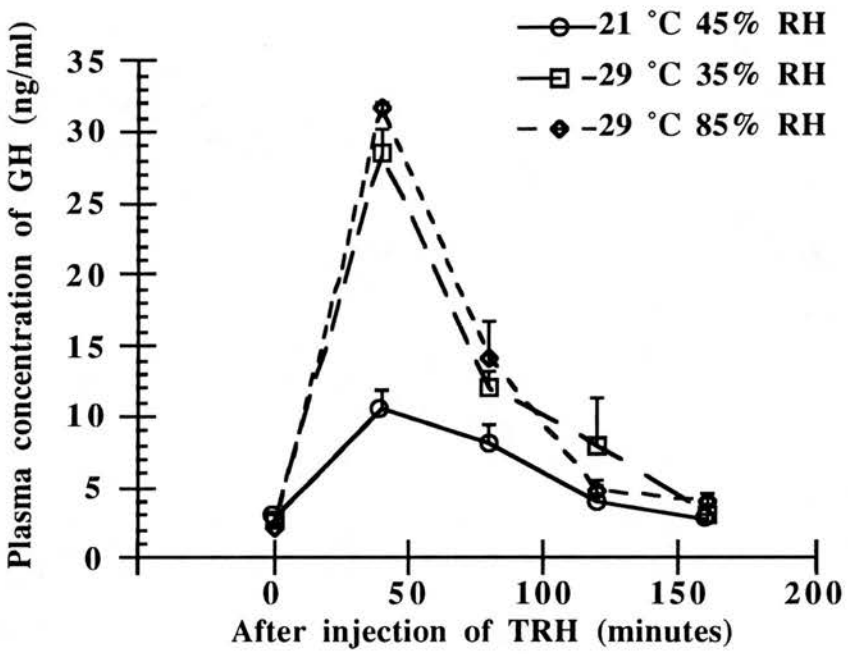


Figure 48. The effects of different thermal loads upon plasma GH responses to the injection of TRH. Values are expressed as means ± SE for six female broiler chickens in Trial One.

Figure 49. The effects of different thermal loads upon plasma T3 responses to the injection of TRH in the second, the third and the fourth trials. Values are expressed as means ± SE.

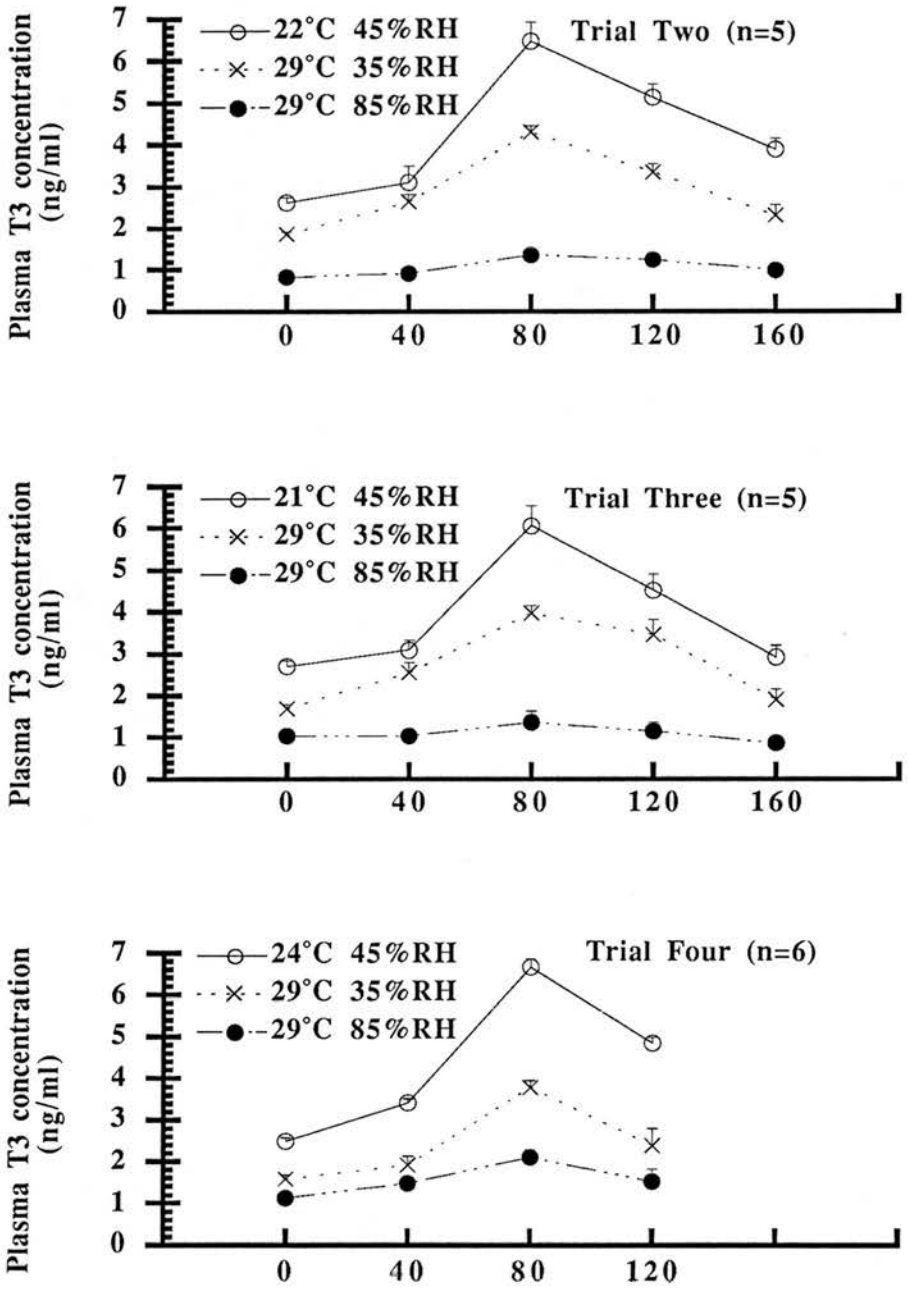
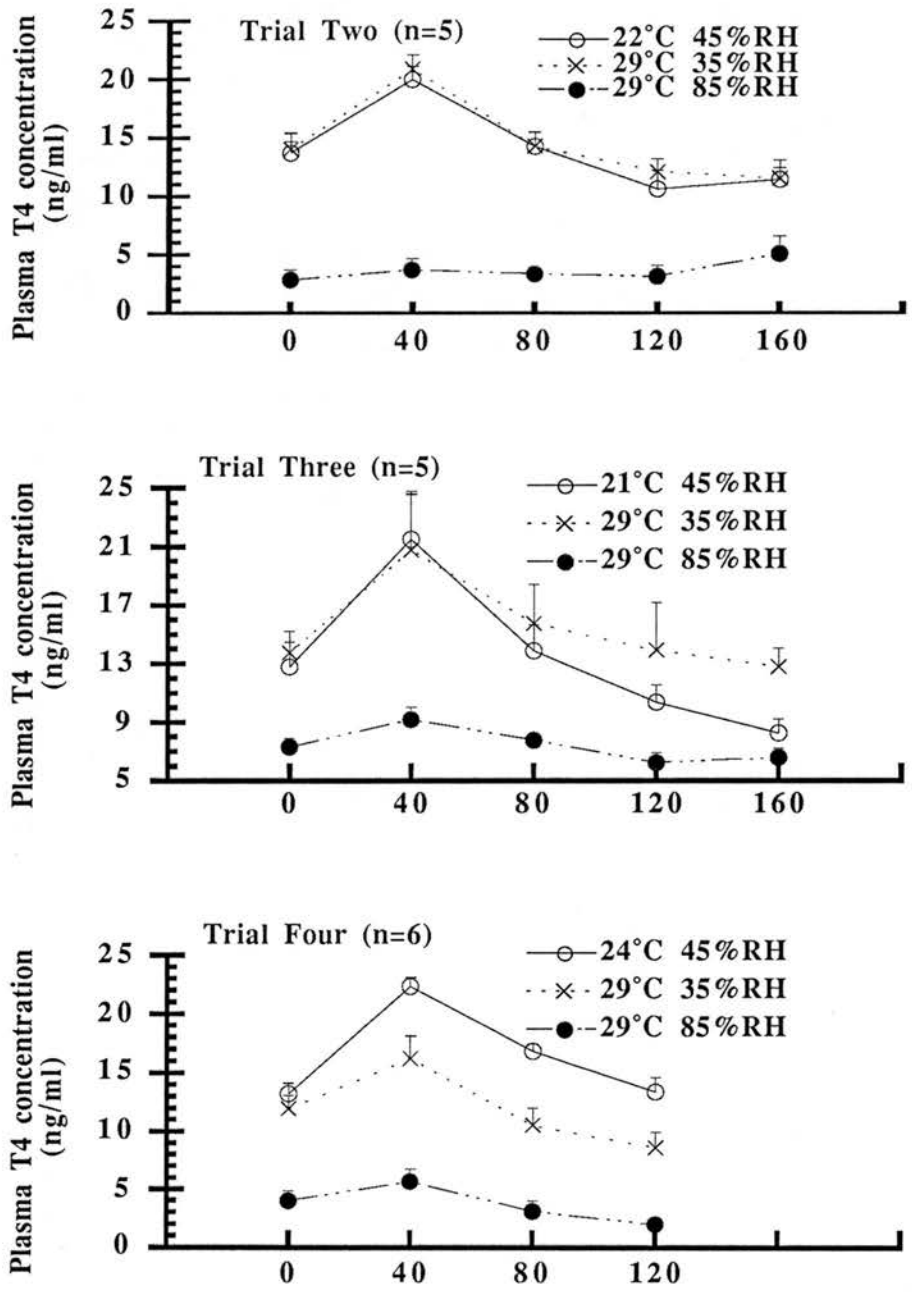


Figure 50. The effects of different thermal loads upon plasma T4 responses to the injection of TRH in the second, the third and the fourth trials. Values are expressed as means ± SE.



Since chronic severe and moderate heat stress treatments change thyroid hormone and GH levels response to TRH in different ways, a changed interaction of the thyrotrophic-somatrophic axis can be explained. Chronic severe and moderate heat stress condition may result in the same way in a 3 fold increase in ($p<0.001$) GH secretion (Figure 48) in response to the TRH induced stimulation of the anterior pituitary gland. Chronic severe and moderate heat stress condition result in different T4 responses to TSH in the thyroid gland and / or TSH response to TRH in the anterior pituitary gland because the T4 response was greatly decreased by severe heat stress but was unaltered by the moderate treatment despite elevated absolute concentrations in Trial One (Figures 47 and 50). In chronic severe and moderate heat stress conditions, stimulation of peripheral T3 production by TRH is inhibited proportionally by heat load despite an increased GH response. It is suggested that this reflects a dissociation of GH from control of 5'-monodeiodination in the liver. The T4 response was greatly decreased by severe heat stress but was unaltered by the moderate treatment despite elevated absolute concentrations (Figures 47 and 50), suggests that TRH response to lower T3 feedback in the hypothalamus is inhibited by severe heat stress conditions but not by the moderate heat stress conditions.

The inhibition of thyroid hormones and abolition of their response to TRH in chronic severe heat stressed broiler chickens were associated with increases of humidity. The results indicated that when assessing the effects of "hot" climates or heat stress upon growth in chickens, the environments should be characterised in terms of their potential influence upon thermal exchange and thermoregulation and not simply by measurement of dry bulb temperature.

5.3. Discussion

5.3.1. The relationship between changes of the peripheral T4, T3 and GH concentrations in chronic severe heat stressed broiler chickens and ambient apparent equivalent temperatures (AET)

The influences upon changes in body temperatures (Tb) and hormonal concentrations in chronic heat stressed chickens by ambient apparent equivalent temperatures (AET) or room temperatures (dry bulb temperatures) are summarised in figures 51-54. Each observation represent means obtained from broilers of all experiments mentioned in the thesis, including those in experiments not yet described.

These results indicate that when assessing the effects of "hot" climates or heat stress upon endocrine function in chickens, the environments should be characterised in terms of their potential influence upon thermal exchange and thermoregulation and not simply by measurement of dry bulb temperature (Figure 51). Otherwise, the result of heat stress upon endocrine function in chickens would not predictable as indicated by the reports of the effects of heat stress on thyroid hormones in the existing literature (Figures 52 and 53, and also see Figure 28 in Section 4.1, Page 140 for details).

Heat stress is usually described in relation to elevated dry bulb temperatures but water vapour density (VD) also has important effects upon heat exchange. The combined effect of dry bulb temperature with water vapour density is known as apparent equivalent temperatures (AET). Figure 51 shows that as ambient AET increases, body temperatures increases. A similar result was also found in room (dry bulb) temperatures against body temperatures, but a bigger variability of body temperature was found at 29 °C. The variability might be the result of different humidities in different experiments. Room temperatures can not correctly predict the

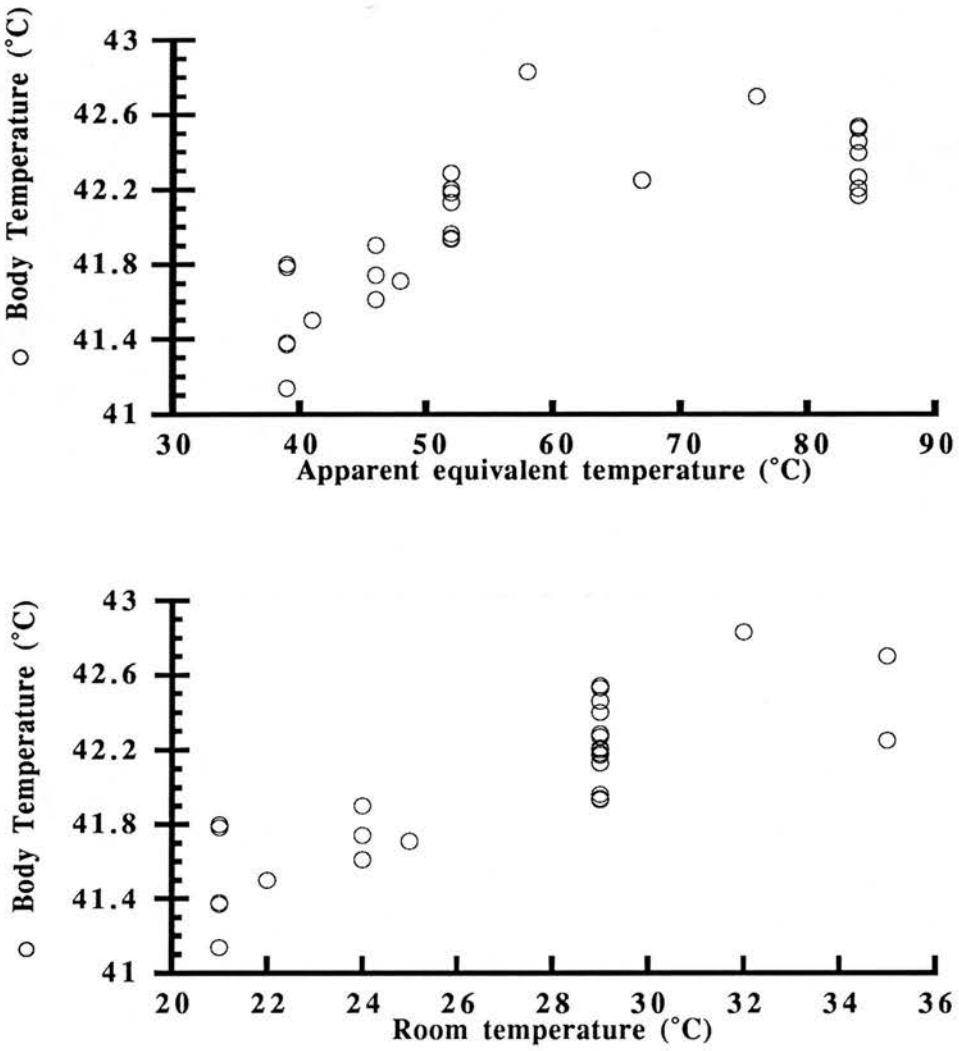


Figure 51. The relationship between body temperatures (Tb), and ambient apparent equivalent temperatures or room temperatures.

The regression equation is:

Tb = 40.9 + 0.0196 AET **or Tb = 39.6 + 0.0883 RoomTemperature**

Predictor	Coef	Stdev	t-ratio	p
Constant	40.8732	0.2140	190.96	0.000
AET	0.019604	0.003694	5.31	0.000

s = 0.3063 R-sq = 54.0% R-sq(adj) = 52.1%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	2.6425	2.6425	28.16	0.000
Error	24	2.2519	0.0938		
Total	25	4.8944			

Predictor	Coef	Stdev	t-ratio	p
Constant	39.6039	0.2816	140.65	0.000
RoomTemp	0.08827	0.01040	8.49	0.000

s = 0.2258 R-sq = 75.0% R-sq(adj) = 74.0%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	3.6708	3.6708	72.01	0.000
Error	24	1.2235	0.0510		
Total	25	4.8944			

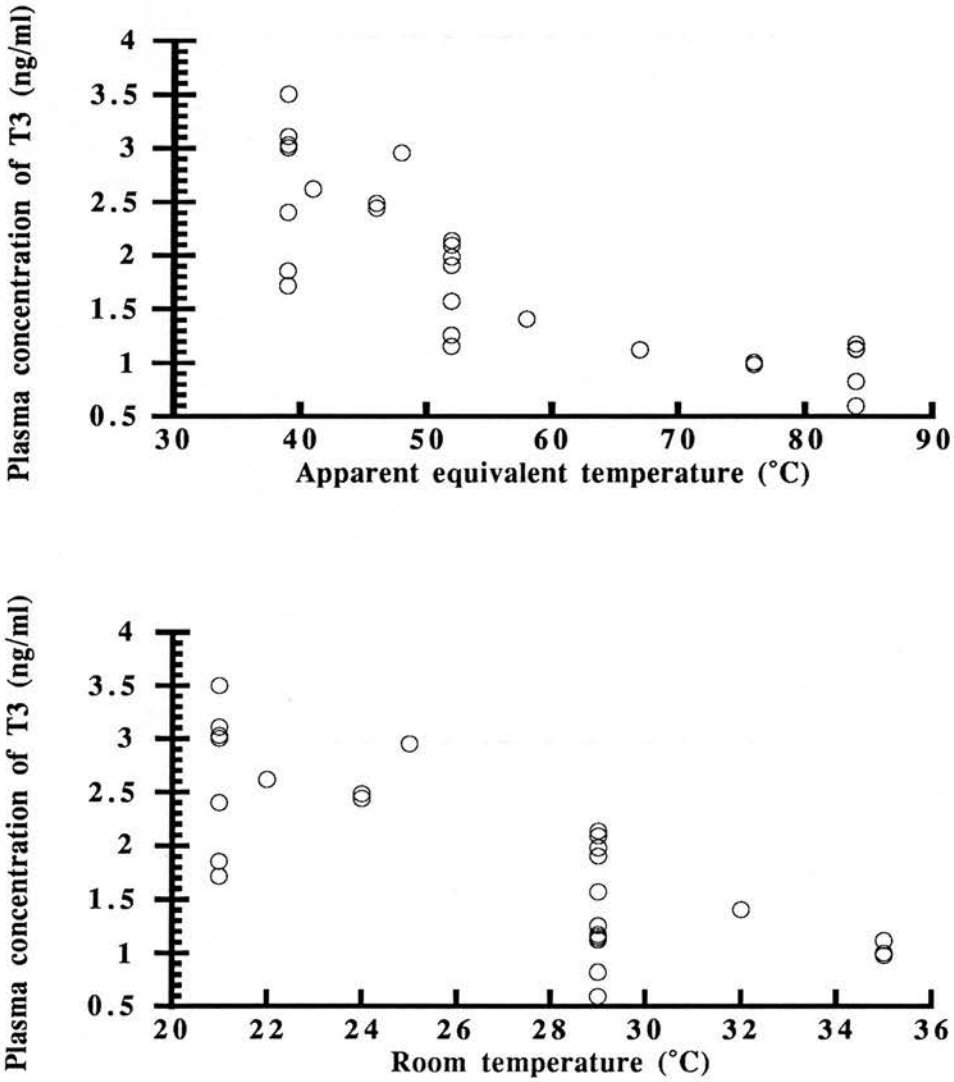


Figure 52. The plasma T3 concentrations in chickens is inhibited proportionally by heat load.

The regression equation is:

T3 = 4.07 - 0.0391 AET or **T3 = 5.46 - 0.133 RoomTemperature**

Predictor	Coef	Stdev	t-ratio	p
Constant	4.0660	0.3053	13.32	0.000
AET	-0.039057	0.005303	-7.36	0.000

s = 0.4695 R-sq = 66.8% R-sq(adj) = 65.5%

Analysis of Variance

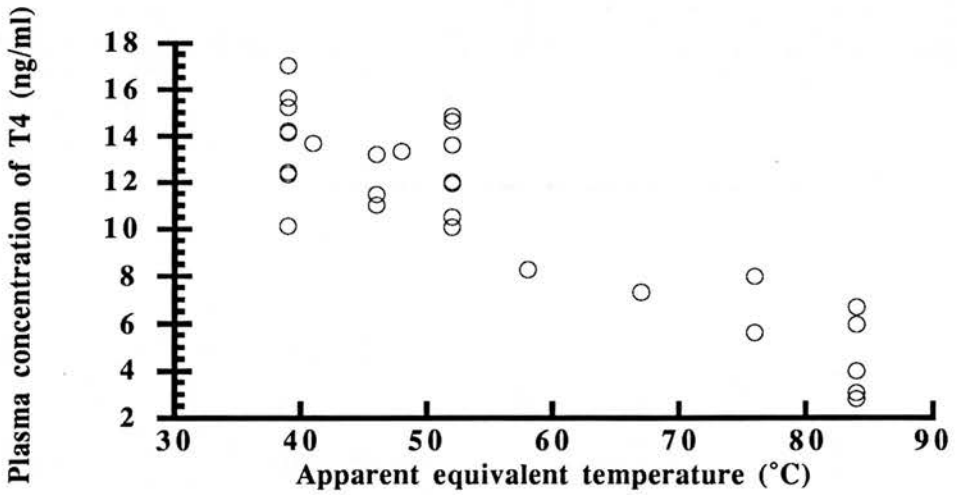
SOURCE	DF	SS	MS	F	p
Regression	1	11.957	11.957	54.24	0.000
Error	27	5.952	0.220		
Total	28	17.909			

Predictor	Coef	Stdev	t-ratio	p
Constant	5.4559	0.5665	9.63	0.000
RoomTemp	-0.13316	0.02097	-6.35	0.000

s = 0.5158 R-sq = 59.9% R-sq(adj) = 58.4%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	10.724	10.724	40.30	0.000
Error	27	7.184	0.266		
Total	28	17.909			



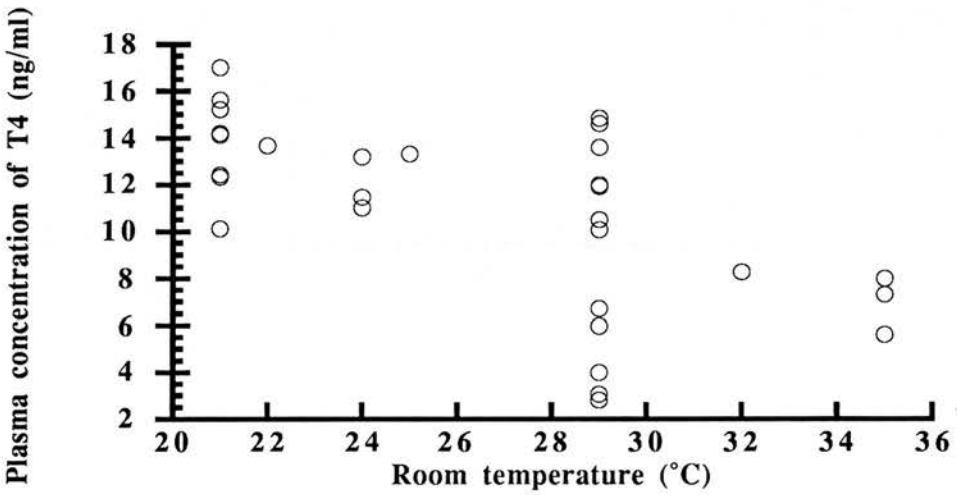


Figure 53. The relationship between plasma T4 concentration in chicken and elevated ambient apparent equivalent temperatures or dry bulb temperatures.

The regression equation is:

T4 = 22.4 - 0.210 AET **or** **T4 = 24.9 - 0.530 RoomTemperature**

Predictor	Coef	Stdev	t-ratio	p
Constant	22.375	1.184	18.90	0.000
AET	-0.21004	0.02056	-10.22	0.000

s = 1.820 R-sq = 79.4% R-sq(adj) = 78.7%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	345.81	345.81	104.38	0.000
Error	27	89.45	3.31		
Total	28	435.26			

Predictor	Coef	Stdev	t-ratio	p
Constant	24.887	3.444	7.23	0.000
RoomTemp	-0.5297	0.1275	-4.15	0.000

s = 3.136 R-sq = 39.0% R-sq(adj) = 36.7%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	169.71	169.71	17.26	0.000
Error	27	265.54	9.83		
Total	28	435.26			

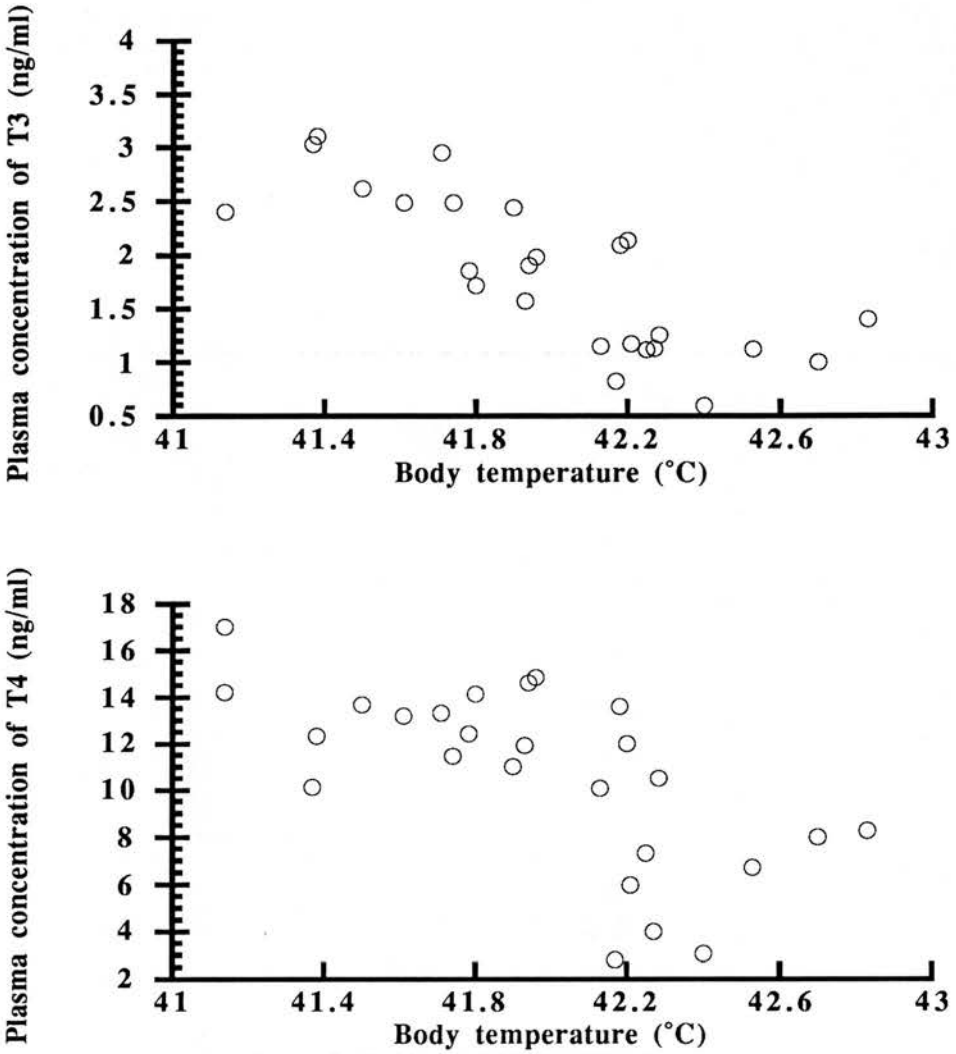


Figure 54. The relationship between changes of the peripheral T3 or T4 concentrations and body temperatures (Tb) in chronic heat stressed chickens

The regression equation is:

T3 = 56.0 - 1.29 Tb **and** **T4 = 247 - 5.63 Tb**

Predictor	Coef	Stdev	t-ratio	p
Constant	56.012	8.709	6.43	0.000
Tb	-1.2908	0.2075	-6.22	0.000

s = 0.4591 R-sq = 61.7% R-sq(adj) = 60.1%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	8.1554	8.1554	38.69	0.000
Error	24	5.0587	0.2108		
Total	25	13.2141			

Predictor	Coef	Stdev	t-ratio	p
Constant	246.98	56.09	4.40	0.000
Tb	-5.632	1.336	-4.21	0.000

s = 2.957 R-sq = 42.5% R-sq(adj) = 40.1%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	155.26	155.26	17.76	0.000
Error	24	209.81	8.74		
Total	25	365.08			

proportional increases of AET which represent heat loads, when the dry bulb (room) temperatures are plotted against AET (see Figure 17, page 118 for details).

These results also show that in chronic severe and moderate heat stress conditions, the plasma T3 concentration in chickens was inhibited proportionally by heat load, which was defined by ambient apparent equivalent temperatures (Figure 52). As ambient apparent equivalent temperatures increased, plasma T3 concentrations decreased. A similar result was also found when room temperatures was plotted against plasma T3 concentrations, but a bigger variability was found at 29 °C (Figure 52).

Figure 53 provides evidence to explain that the cause of the conflicting results relating to T4 responses in heat stressed chickens. The conflicting results may be due to the elevated dry bulb temperatures. Plasma T4 concentration in chickens seems associated with elevated ambient apparent equivalent temperatures, but not dry bulb temperatures. As ambient apparent equivalent temperatures increases, plasma T4 concentrations decreased. When room temperatures (under 30 °C) was plotted against plasma T4 concentrations, however, there was a much weaker relationship between the changes of plasma T4 concentrations and the increases of room temperatures (Figure 53).

5.3.2. The relationship between changes of the peripheral T4, T3 and GH concentrations and body temperatures in chronic severe heat stressed broiler chickens

The definition of chronic severe and moderate heat stress conditions may be identified by the relationship between changes of the peripheral T3 and T4 concentrations, and body temperatures in chronic heat stressed chickens (Figure 54).

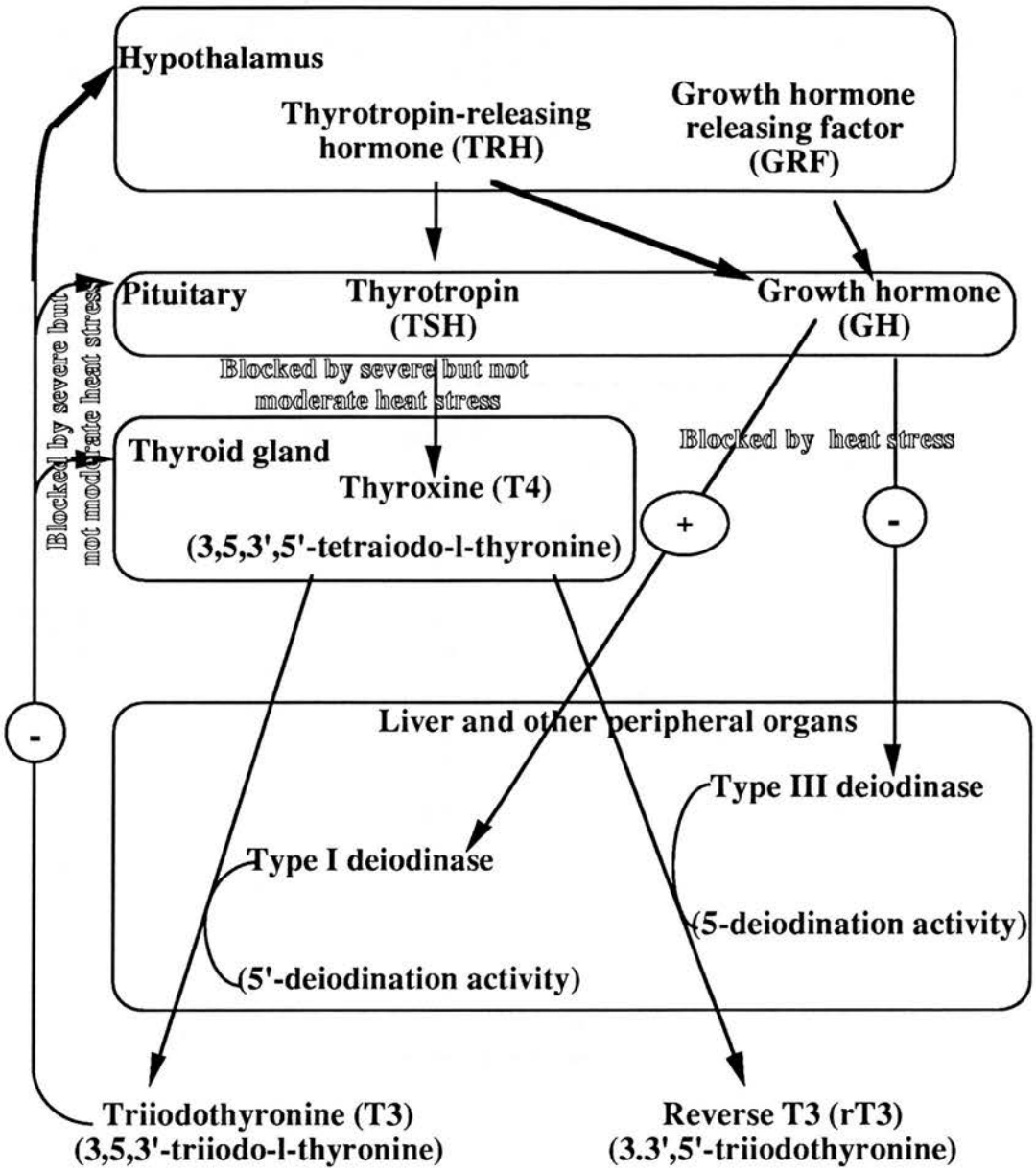
5.4. Conclusion

In chronic severe and moderate heat stress conditions, stimulation of peripheral T3 production by TRH is inhibited proportionally by heat load despite an increased GH response. Chronic severe and moderate heat stress conditions may result in different ways response in T4 to TSH in the thyroid gland and / or TSH response to TRH in the anterior pituitary gland because the T4 response was greatly decreased by severe heat stress but was unaltered by the moderate treatment despite elevated absolute concentrations (see Figure 55 for details). Other factors, such as chicken type I hepatic deiodinase and type III hepatic deiodinase regulated by GH, may also be affected by heat stress in the conversion of T4 (thyroxine) into T3 (3,3',5-tri-iodothyronine), the major calorigenically activity hormone which can act on most tissues to increase metabolic activity and protein synthesis, or reverse T3 (rT3, 3,3',5'-tri-iodothyronine).

Though the exact mechanisms controlling thyroid function of heat stressed chickens are still poorly understood, they are unlikely to be due to the influence of feed intake only as originally proposed. Moreover, the changes of the levels of T4 and T3 in chickens exposed to heat stress condition may indicate reduction in hepatic 5'-monodeiodination.

These results also suggest that stimulation of peripheral T3 production by TRH is inhibited proportionally by heat load rather than temperature *per se* despite an increased GH response. Although chronic heat stress condition by itself hardly showed any effect on pre-injection GH values, both chronic severe and moderate heat stress conditions enhanced both the GH secretory response to TRH and the GH clearance. The apparent discrepancy in the relationship between circulating GH concentrations versus growth for the chronic heat stressed birds likely results from a

Figure 55. Modifications to the hypothalamus-pituitary-thyroid axis and the hypothalamus-pituitary-somatotroph-liver axis in poultry by moderate and severe heat stress.



T3 being derived almost exclusively from the peripheral conversion of T4. This reaction is stimulated by GH when chickens in thermoneutral temperatures environment. GH regulates the peripheral conversion of T4 to T3 by the means of stimulation of the liver type I deiodinase (5'-monodeiodination) activity or inhibition of the liver type III deiodinase (5-monodeiodination) activity when chickens in thermoneutral temperatures environment. The normal functions of the hypothalamus-pituitary-thyroid axis may regulate by the negative feedback of T3.

Chronic heat stress reduces circulating T3, possibly by a reduction in hepatic 5'-monodeiodination. Stimulation of T4 production by negative feedback of peripheral low T3 concentration is blocked by severe but not moderate heat stress condition.

defect in tissue sensitivity to GH, rather than reflecting the true relationship of GH to growth in poultry. Hepatic GH binding might be lower in the chronic heat stressed birds compared with control broilers. A deficiency of hepatic GH receptors in the chronic heat stressed birds was supported by the failure of GH to increase plasma T3 concentrations. The increased magnitude of GH peak following TRH administration under both chronic severe and moderate heat stress conditions may be explicable in terms of the dissociation of GH from control of 5'-monodeiodination, or the depressive effects of thyroid hormones on GH secretion (Harvey *et al.*, 1983; Harvey, 1983; Harvey, 1990b, Harvey *et al.*, 1991a) while the thyroid function is known to be reduced in warmer environments (May, 1978; May and McNaughton, 1980; Cogburn and Harrison, 1980; Klandorf, 1982; Cogburn and Freeman, 1984; May *et al.*, 1986; Mitchell and Goddard, 1990; Mitchell and Carlisle, 1992; Iqbal *et al.*, 1990, 1987; Kühn *et al.*, 1984). The reduced thyroid function may then result in an enhanced sensitivity of GH to a secretagogue challenge such as TRH (Harvey *et al.*, 1990a). The enhanced GH decrease between 40 and 80 minutes after a similar TRH injection in the chronic heat stressed birds should be interpreted carefully.

It may be interpreted as a shorter secretagogue challenge, due to a hypothetical shorter half-life of TRH while an increased half-life of GH in chronic heat stress condition reared chicks, or to an increased secretion of GH. From the higher GH peak values obtained after TRH linked with a reduced thyroid function on a TRH challenge and the higher GH levels at a post-injection time interval of 80 minutes, the first possibility seems valid. The second possibility is rather unlikely in view of the higher decrease in GH levels at a specific time interval and the acute elimination rates calculated as the differential from the individual hormone disappearance curves. It has been suggested that animals utilising somatotrophin faster will exhibit lower circulating somatotrophin levels (Siers and Swiger, 1971). Pigs selected for heavier weights at a given age have a higher rate of metabolic clearance of somatotrophin

(Arbona *et al.*, 1988), and young, growing chickens have a higher metabolic clearance of GH per unit of metabolic weight than adults (Lauterio and Scanes, 1988). Nevertheless, the paradox of lower circulating GH in association with faster growth or larger body size when comparisons are made between the thermoneutral and heat stressed birds may be unique to the observation with many species including poultry and animals for which a large growth-promoting effect of GH has been demonstrated. When growth-selected lines are compared with slower growing randombred or layer strains, the slower growing lines generally display higher circulating GH concentrations in chickens (see Section 1.4, page 20 for details).

In chronic heat stress condition reared chickens, growth rate is depressed and plasma T3 levels are reduced despite elevated GH concentrations. This apparent dissociation of GH from control of 5'-monodeiodination might be attributed to a decreased capacity of GH receptors as chickens possessing the sex-linked dwarf gene (SLD) (Kühn *et al.*, 1989). A reciprocal relationship between T3 and GH has also been reported in chickens whose growth rate was depressed by chickens possessing the sex-linked dwarf gene (SLD) (Lam *et al.*, 1989) and restriction of energy and protein intake (Rosebrough *et al.*, 1989).

It has been established that GH secretion in poultry is episodic, with high-concentration peaks or pulses occurring at regular intervals of approximately 60 to 90 min, superimposed on a relatively constant baseline (Scanes *et al.*, 1983; Cartwright *et al.*, 1984; Buonomo *et al.*, 1984a,b; Vasilatos-Younken and Leach, 1986; Shaw *et al.*, 1987). Thus, measures of plasma baseline GH concentration for between the thermoneutral and heat stressed birds comparisons could not be obtained coincidentally or reliably because of a GH pulsatile pattern. The magnitude of GH secretion is influenced primarily by the amplitude of secretory pulses and baseline level (i.e., versus pulse frequency or other pattern components). A higher GH

secretory response to TRH was observed in the heat stressed birds, which may be linked to the higher endogenous pulsatility. This interpretation of the higher endogenous pulsatility in chronic heat stressed chickens might be wrong. Within lines, secretory pattern characteristics and, in particular, pulse amplitude tend to reflect growth. Experimentally induced or natural GH pulses have been found to be related to growth. There is considerable evidence to suggest that the pattern of plasma somatotrophin influences body growth factors in mammalian species such as the rat and human (Robinson and Clark, 1987; Jansson *et al.*, 1989). Maintenance of a high-amplitude, low-baseline pattern of GH secretion may be essential for effective biological action of the hormone in growing poultry as well. When cGH was administered to late post-hatch (8 to 11 wk) broiler pullets by means of intravenous infusion in either a continuous pattern or a pulsatile pattern (to mimic the frequency and pulse amplitude of 2-wk-old, rapidly growing chicks) growth rate and feed efficiency were improved with pulsatile cGH, whereas growth rate was lower and feed efficiency significantly reduced by continuous administration of cGH, relative to controls (Vasilatos-Younken *et al.*, 1988b).

In conclusion then, 1) Chronic heat stress reduces circulating T3, possibly by a reduction in hepatic 5'-monodeiodination, an effect which is proportional to heat load rather than temperature *per se*. 2) Stimulation of peripheral T3 production by TRH is inhibited proportionally by heat load despite an increased GH response. It is suggested that this reflects a dissociation of GH from control of 5'-monodeiodination. 3) Stimulation of T4 production by negative feedback of low peripheral T3 concentration is inhibited under severe heat stress conditions but not under moderate heat stress conditions. Peripheral T4 concentration was, therefore, decreased at severe heat stress conditions but increased or unaltered at moderate heat stress conditions. 4) Changes in the control of thyroid hormone metabolism during chronic exposure to high environmental temperatures may, at least in part, account for the reduction in

growth rate observed under these conditions. These effects may be related to the extent of hyperthermia induced by a specific heat load but are not the consequence of concomitant decreases in food intake. 5) When assessing the effects of "hot" climates or heat stress upon endocrine function and growth in chickens, the environments should be characterised in terms of their potential influence upon thermal exchange and thermoregulation and not simply by measurement of dry bulb temperature. 6) Chickens may be able to divide high ambient temperatures into moderate and severe heat stress conditions.

Chapter six

The thyroid hormonal profiles in response to strategies for reduction or alleviation of heat stress and TRH challenge in the domestic fowl

6.1. The domestic fowl with the naked neck gene and their response to heat load and TRH challenge

6.1.1. Introduction

The reported differences in T4 and T3 in responses in heat stressed chickens in the existing literature may be a result of different breeds and strains employed in each study because the genetic constitution of the layer breed with smaller body size allows more control over their thermoregulation than the heavy breed (see Washburn, 1985 for review; Washburn *et al.*, 1992). The previous experiments have also demonstrated that chronically severe heat stressed birds (58, 66, 84 AET) reduced their growth rate and also decreased plasma concentrations of T3, T4 while GH levels increased significantly. Stimulation of 5'-monodeiodination by TRH (or chicken GH) was greatly attenuated in heat stressed birds but not thermoneutral controls.

In this section the responses of the hypothalamus, the anterior pituitary gland, the thyroid gland and the 5'-monodeiodination of the domestic fowl with or without the naked neck gene to elevated exogenously administered thyrotrophin releasing hormone (TRH) *in vivo* have been examined in terms of the effects on plasma concentrations of thyroxine, tri-iodothyronine in order to establish the thyroid

hormonal basis of the domestic fowl with the naked neck gene and their response to humid heat stress condition. The stock of immature female broiler chicks with naked neck gene (Na/Na) were originally obtained from Holland in earlier 1980's. They were relax-bred by inter-cross for several generations in the Institute. Their body weights were, therefore, lower than modern broiler female chickens' body weights at the same age.

As we are aware, thyroid hormones and GH are involved in the metabolic thermoregulation of poultry. To our knowledge, the thyroid hormonal profile of the 10-year randombred line of domestic fowl with the naked neck gene is not yet known. Although it is clear that pituitary and thyroid hormone can play an important role in control of molt in hens, no complete picture of the relationships between naked neck birds and normal (commercial) broilers is available until now.

To our knowledge, the physiological differences of birds with and without naked neck gene are not yet known. This information is required to predict any additional consequences of selection for a heat stress resistant line birds. However, thyroid hormone levels of the naked neck birds, which have less feather cover, and a better performance in high environmental temperature (31 °C) and a consistently greater survival rate to heat stress (Mérat, 1980; Cahaner *et al.*, 1992, 1993), may be different from normal birds.

In order to test the function of the hypothalamo-pituitary-thyroid axis and the thyrotrophic-somatrophic axis, and to examine their response to TRH *in vivo* in female broilers (Mitchell, 1987b), we carried out this experiment to investigate the thyroid hormonal basis of decreased growth rate in the domestic fowl with the naked neck gene and their response to humid heat stress condition. It was therefore considered appropriate to study the plasma thyroid hormone concentrations and their

responses to exogenously administered TRH *in vivo* during chronic heat stress in normal (commercial) and naked neck chickens between 3 and 6 weeks of age.

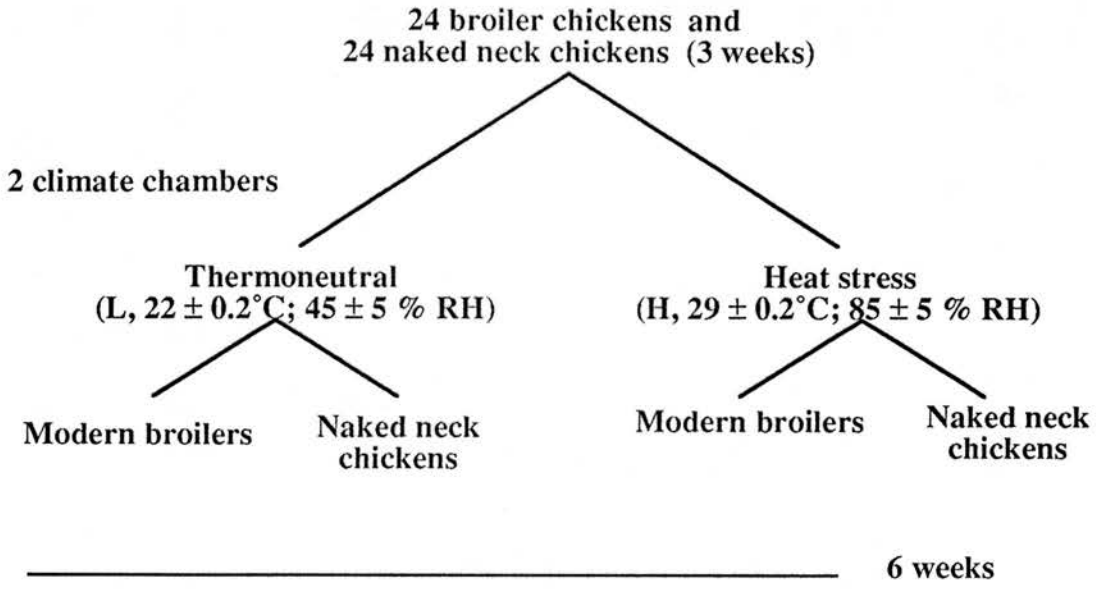
6.1.2. Experimental procedure

Both modern broilers and naked neck chicks were maintained in brooders until 21 days old and vaccinated for Gumboro at age of 7 days. They were numbered using wing bands and were maintained on a 14L: 10D photoperiod in the brooder. The body weights of immature female modern broiler chicks (na/na) were heavier than of naked neck chicks (Na/Na) at the age of 21 days.

All experiments were performed on individually caged birds in climate chambers in which both temperature and relative humidity were accurately controlled (±0.2 °C; ±5% RH). They were maintained on a lighting schedule of 23L: 1D with food and water freely available. The birds were reared in wire cages and were exposed to each of the 2 thermal loads for 24 days from 21 to 44 days of age (see Figure 56 for details). Control birds were maintained at 22 °C and 45% RH. Severe chronic heat stressed birds (HHS) were maintained at 29 °C and 85% RH. Plasma samples were obtained at 11:00 am from 5 birds by venipuncture (brachial vein) in each treatment at the end of this period. At the end of exposure, further samples were taken immediately prior to and at 40 minute intervals following the subcutaneous administration of TRH (10 μg/kg body weight). In all 12 birds on each treatment, body temperature and body weight were determined every other day. Plasma concentrations of thyroxine (T4) and tri-iodothyronine (T3) were determined by radioimmunoassay (see Section 2.3.1, Page 114 for details).

Data from experiments performed as balanced designs were analysed statistically by one-way and two-way analysis of variance considering treatments groups within each temperature. Differences among least square means were estimated

Figure 56. Diagram of experimental procedure



Blood samples (0)

Inject TRH (s.c.) 10 μg kg-1

Blood samples at 40, 80, 120 and 160 minutes post injection

using the Student un-pair t-test for these values. Statistics were calculated using MINITAB and Hewlett-Packard General Statistics Pac.

6.1.3. Results

The comparisons of the plasma thyroid hormone concentrations and their responses to exogenously administered TRH *in vivo* during chronic heat stress in commercial and naked neck chickens are summarised in figures 57-63.

The 24-day chronic heat stress treatments had pronounced effects on the body temperature (Figure 57), body weight (Figure 58) and body weight gain (Figure 59) in modern broilers but not those with naked neck gene. Rectal temperatures, used as a measure of response, were all within the normal range at heat stress treatment in the 10-year randombred broilers with naked neck gene while at the same heat stress treatment the commercial modern broiler chickens showed a significant rise of rectal temperature ($p<0.001$ - Figure 57). The naked neck chickens have significantly lower body weight ($p<0.001$ - Figure 58) and significantly lower body weight gain ($p<0.001$ - Figure 59) than modern broilers throughout this period. The modern broilers at heat stress treatment showed a significant inhibition of body weight (88%) ($p<0.01$ - Figure 58) and body weight gain (82%) ($p<0.01$ - Figure 59).

The 24-day chronic heat stress treatments had a different effect on the plasma baseline concentrations of thyroid hormones and their response to TRH between the 10-year randombred broilers with naked neck gene and modern broiler chickens. Plasma T4 was reduced in modern broilers ($p<0.001$ - Figure 60) subject to chronic heat stress but plasma T4 was increased in naked neck chickens ($p<0.05$ - Figure 60) subject to chronic heat stress ($p<0.01$) during the 3-week of exposure compared to their thermoneutral control birds, although there was a significant lower plasma T4 concentration in naked neck chickens ($p<0.05$ - Figure 60) in comparison with

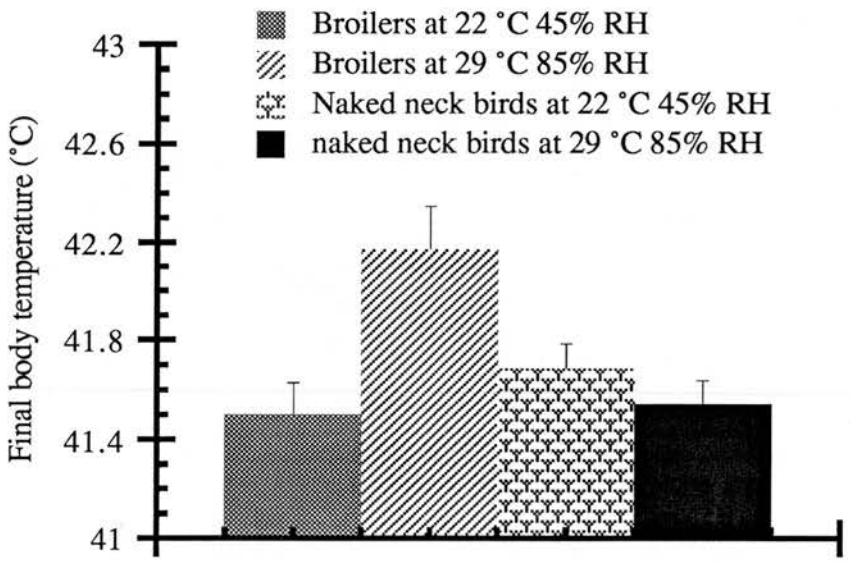


Figure 57. Comparison of the body temperature between naked neck birds and modern broilers at thermoneutral (22 °C 45% RH) and severe heat load (29 °C 85% RH) condition. Values are expressed as means ± SEM for twelve female broiler chickens.

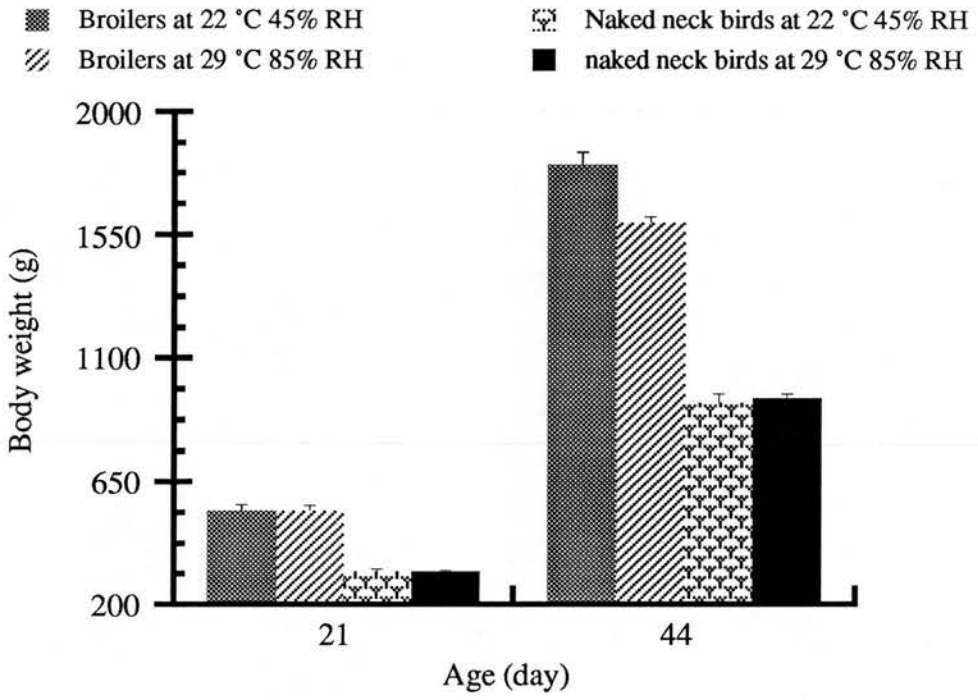


Figure 58. Comparison of the body weight between naked neck birds and modern broilers at thermoneutral (22 °C 45% RH) and severe heat load (29 °C 85% RH) condition. Values are expressed as means ± SEM for twelve female broiler chickens.

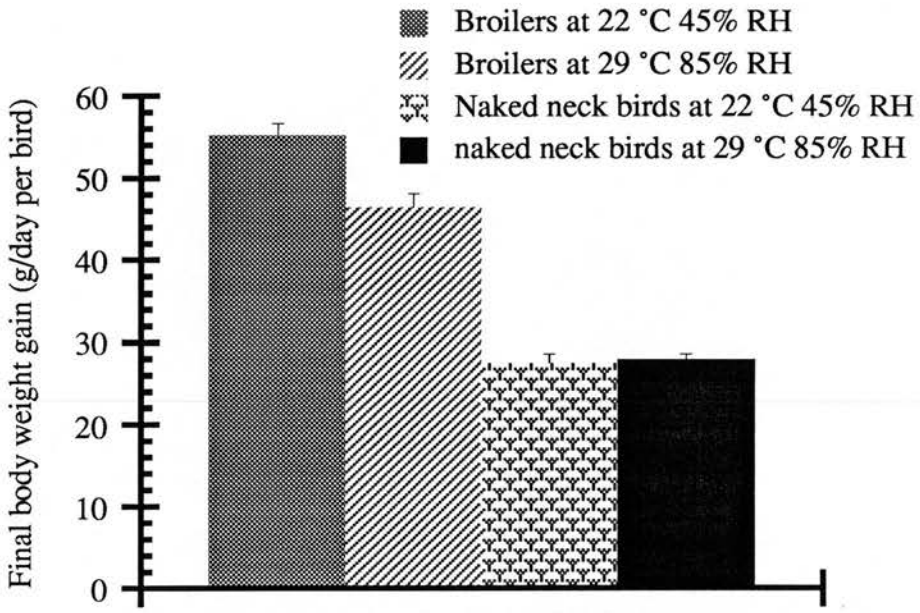


Figure 59. Comparison of the body weight gain between naked neck birds and modern broilers at thermoneutral (22 °C 45% RH) and severe heat load (29 °C 85% RH) condition. Values are expressed as means ± SEM for twelve female broiler chickens.

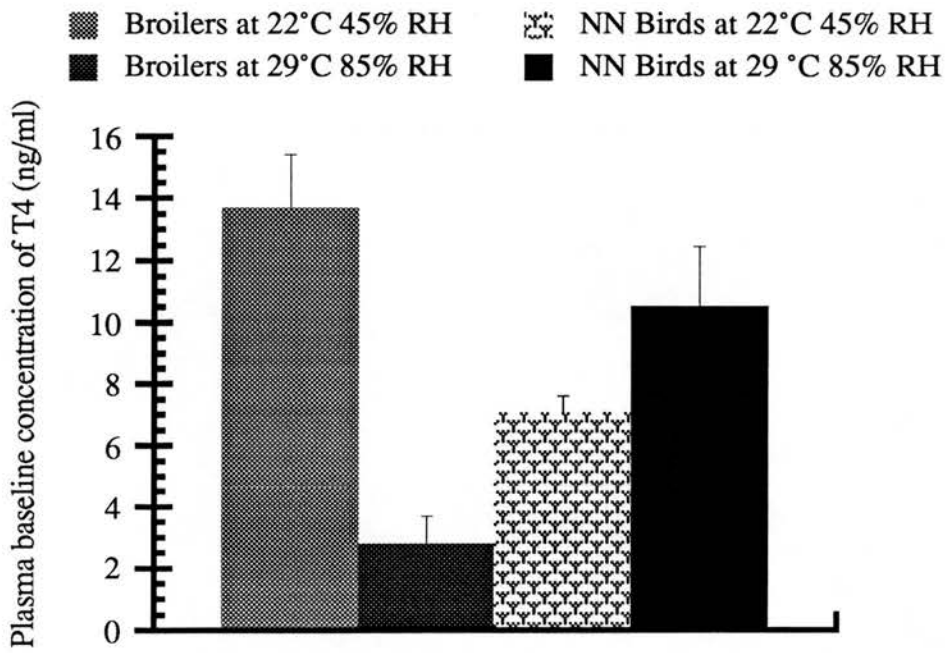


Figure 60. Comparison of the baseline plasma T4 concentration (ng/ml) between naked neck (NN) birds and modern broilers at thermoneutral (22 °C 45% RH) and severe heat load (29 °C 85% RH) condition. Values are expressed as means ± SEM for five female broiler chickens.

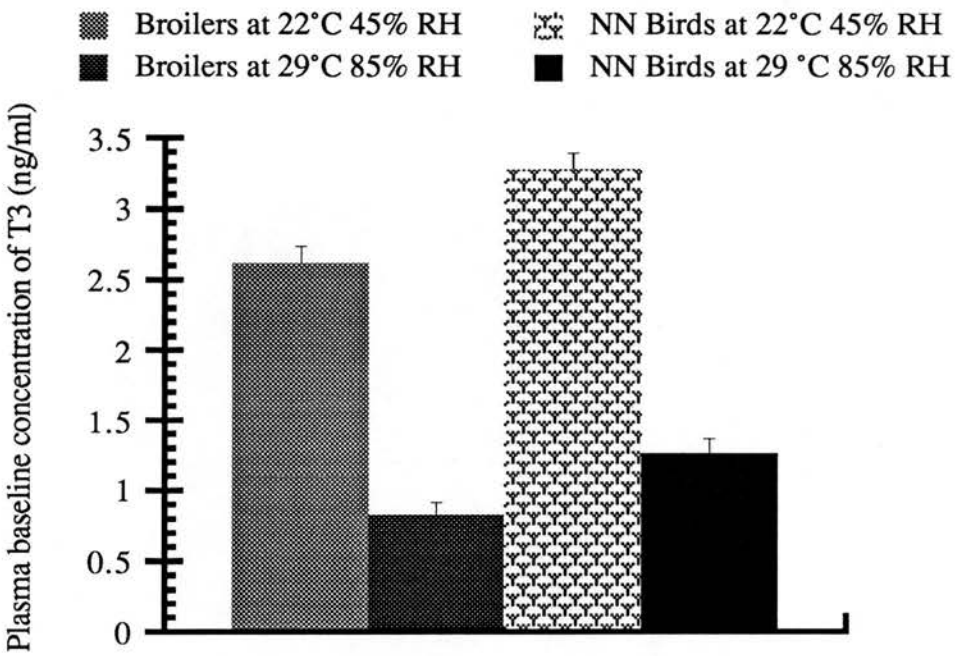


Figure 61. Comparison of the baseline plasma T3 concentration (ng/ml) between naked neck (NN) birds and modern broilers at thermoneutral (22 °C 45% RH) and severe heat load (29 °C 85% RH) condition. Values are expressed as means ± SEM for five female broiler chickens.

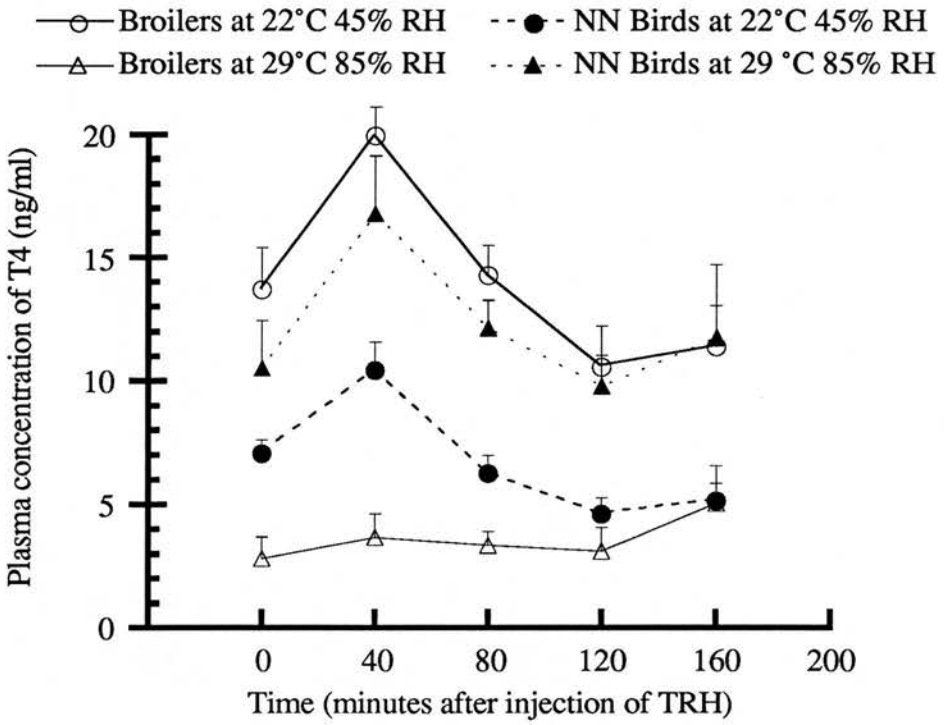


Figure 62. Comparison of peripheral T4 concentration (ng/ml) responses to TRH injections (10 μg/kg body weight) between naked neck (NN) birds and modern broilers at thermoneutral (22 °C 45% RH) and severe heat load (29 °C 85% RH) condition. Values are expressed as means ± SEM for five female broiler chickens.

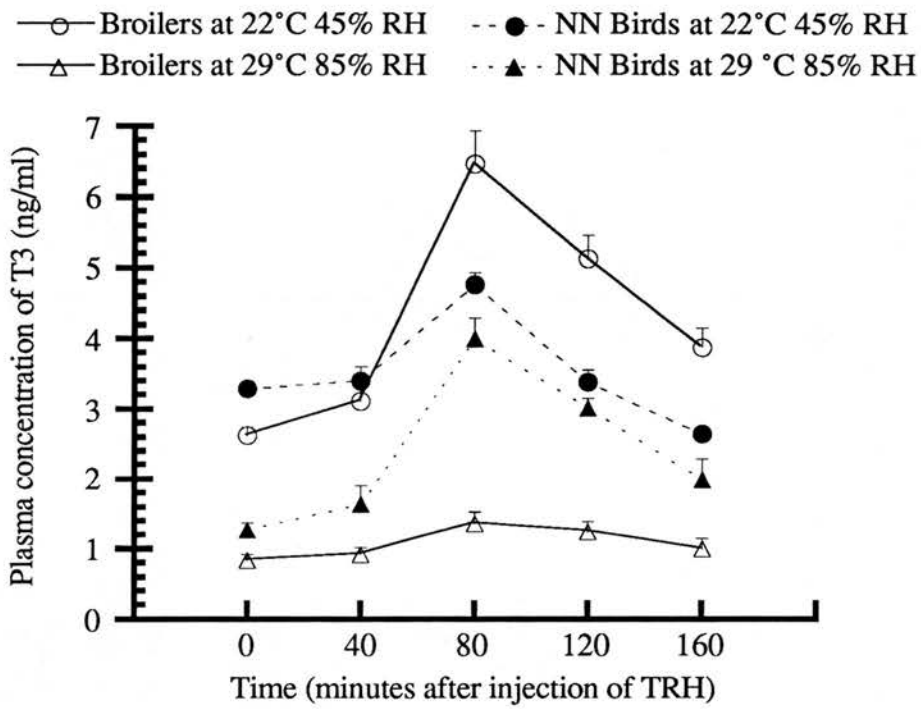


Figure 63. Comparison of peripheral T3 concentration (ng/ml) responses to TRH injections (10 µg/kg body weight) between naked neck (NN) birds and modern broilers at thermoneutral (22 °C 45% RH) and severe heat load (29 °C 85% RH) condition. Values are expressed as means ± SEM for five female broiler chickens.

modern broilers at thermoneutral condition. However, a significant higher plasma T3 concentration in naked neck chickens ($p<0.05$ - Figure 61) in comparison with modern broilers was found at both thermoneutral and heat stress conditions. The T4 response to TRH was greatly decreased by heat stress in modern broiler chickens but not those with naked neck gene ($p<0.001$ - Figure 62). The T3 response to TRH was also reduced by heat stress ($p<0.001$ - Figure 63) in modern broiler chickens but not those with naked neck gene. The T3 response to TRH was smaller in naked neck birds at thermoneutral control condition despite elevated absolute concentrations (Figure 63). The T4 response to TRH was also smaller in naked neck birds at thermoneutral control condition (Figure 62).

These results indicate that the plasma T3 concentration in chickens expressing the naked neck gene was always significantly higher ($p<0.05$ - Figure 61) than modern broilers at both thermoneutral and severe heat stress conditions. These results suggested that not only the environments should be characterised in terms of their potential influence upon thermal exchange and thermoregulation but also the breeds should be characterised in terms of their potential influence upon feather condition, growth rate and body weight. Otherwise, the result of "hot" climates or heat stress upon endocrine function and growth in chickens would not be predictable as indicated by the reports of the effects of heat stress on thyroid hormones in the existing literature (see Figure 28 in Section 4.1, Page 140 for details).

These results also suggested that stimulation of peripheral T4 ($p<0.05$ - Figure 62) and peripheral T3 ($p<0.05$ - Figure 63) production by TRH is not inhibited by these hot conditions in naked neck chickens. Although chronic heat stress condition inhibited the peripheral T4 ($p<0.05$ - Figure 62) and peripheral T3 ($p<0.05$ - Figure 63) secretory response to TRH in modern broilers.

Different strains of birds may be able to divide the same heat load into different degrees of heat stress. At 29 °C 85% RH, where modern broiler chickens suffered from severe heat stress condition, naked neck birds appeared to suffer less from heat stress indicated by their normal body temperatures and a higher ($p<0.05$) T4 and lower T3 secretion ($p<0.001$) response to the same condition (Figure 60 and 61). Although chronic severe and moderate heat stress condition may result with different ways in an altered T4 response to TSH in the thyroid gland and / or TSH response to TRH in the anterior pituitary gland because the T4 response was greatly decreased by severe heat stress but was unaltered by the moderate treatment despite elevated absolute concentrations in modern broiler chickens. The consistently enhanced plasma T3 concentration in naked neck chickens whether keeping them at either thermoneutral or hot condition should be interpreted carefully.

It may be interpreted that naked neck birds have a higher thyroid functional state, due to a higher plasma T3 concentration ($p<0.05$ - Figure 61 and 63) compared to modern broilers, or to have a hypothyroid functional state, due to a lower plasma T4 concentration under thermoneutral conditions. Since stimulation of peripheral T3 ($p<0.05$ - Figure 63) production by a TRH challenge is not inhibited by hot condition and T3 is the thyroid hormone affecting heat production in birds, the first possibility seems valid. The second possibility is rather unlikely since T4 is the only pre-hormone on thyroid function of heat production and the higher stimulation of peripheral T4 ($p<0.05$ - Figure 62) production by a TRH challenge is not inhibited by hot condition. It has been suggested that the genetic constitution of the breeds with smaller body size allowed more control over their thermoregulation than heavy breeds (Yeates *et al.*, 1941) due to genetic differences in adaptation to high environmental temperatures (Hutt, 1938) and humidities (Lee *et al.*, 1945). The body temperatures of the breeds with smaller body size response to high environmental temperatures showed less

change in comparison with heavy breeds (Yeates *et al.*, 1941; Lee *et al.*, 1945; Washburn *et al.*, 1992).

6.2. The effects of differing heat loads upon conversion of T4 into T3 in response to TRH are different in naked neck chickens

6.2.1. Introduction

Different breeds respond to the same heat load (29 °C with 85%RH) differentially. However, the effects of different heat loads upon the endocrine responses of broiler chickens with or without naked neck gene have not been investigated.

In this section the responses of the hypothalamus, the anterior pituitary gland, the thyroid gland and the 5'-monodeiodination of the domestic fowl with or without the naked neck gene to elevated exogenously administered thyrotrophin releasing hormone (TRH) have been examined in terms of the effects on plasma concentrations of thyroxine, tri-iodothyronine in order to establish the thyroid hormonal characteristics of the naked neck chickens and their response to different heat stress conditions.

To develop a selection criterion for resistance to heat stress, it is desirable to understand the hormonal basis of response to chronic heat stress. It was therefore considered appropriate to study the effects of chronic different heat loads upon plasma hormone levels and the endocrine responses to TRH injection in broiler chickens with or without naked neck gene during their "rapid growth phase" at period between 3 and 6 weeks of age.

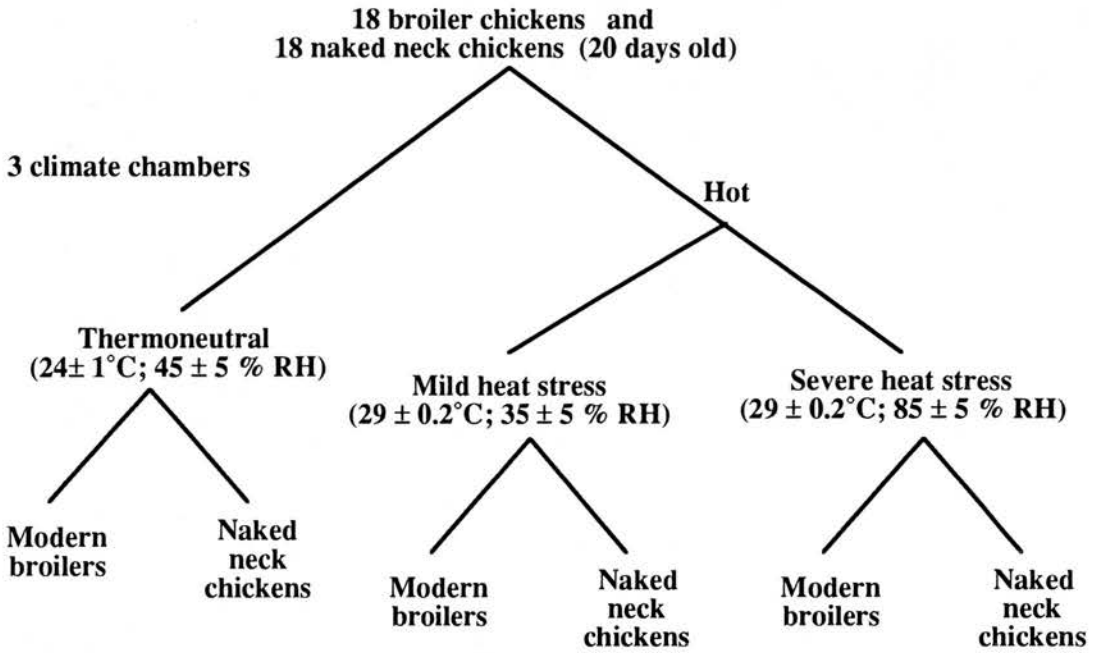
6.2.2. Experimental procedure

Both commercial broiler chicks and the naked neck chicks were maintained in brooders until 21 days old and vaccinated for Gumboro at age of 7 days. They were numbered using wing bands and were maintained on a 14L: 10D photoperiod in the brooder.

All experiments were performed on individually caged birds in climate chambers in which both temperature and relative humidity were accurately controlled (±0.2 °C; ±5% RH). They were maintained on a lighting schedule of 23L: 1D with food and water freely available. The birds were reared in wire cages and were exposed to each of the three thermal loads for 24 days from 21 to 44 days of age (see Figure 64 for details). Control birds were maintained at 24 °C and 45% RH. Moderate chronic heat stressed birds (MHS) were maintained at 29 °C and 35% RH. Severe chronic heat stressed birds (HHS) were maintained at 29 °C and 85% RH. Plasma samples were obtained at 11:00 am from 6 birds by venipuncture (brachial vein) in each treatment at the end of this period. At the end of exposure, further samples were taken immediately prior to and at 40 minute intervals following the subcutaneous administration of TRH (10 μg/kg body weight). In all 6 birds on each treatment, body temperature and body weight were determined every other day. Plasma concentrations of thyroxine (T4) and tri-iodothyronine (T3) were determined by radioimmunoassay (see Section 2.3.1, Page 114 for details).

Data from experiments performed as balanced designs were analysed statistically by one-way and two-way analysis of variance considering treatment groups within each temperature. Differences among least square means were estimated using the Student un-pair t-test for these values. Statistics were calculated using MINITAB and Hewlett-Packard General Statistics Pac.

Figure 64. Diagram of experimental procedure



Blood samples

Inject TRH (s.c.) 10 μg kg-1

Blood samples at 40, 80, 120 and 160 minutes post injection

6.2.3. Results

The comparisons of the plasma thyroid hormone concentrations and their responses to exogenously administered TRH *in vivo* during chronic different heat stress conditions in normal (commercial) and naked neck chickens are summarised in figures 65-70.

The chronic heat stress treatment had pronounced effect on food intake, feed conversion ratio and body weight gain (Figure 65) in modern broilers but not those with naked neck gene. The commercial modern broiler chickens under heat stress treatment showed a significant reduction of body weight gain ($p<0.05$ - Figure 65).

Higher thyroid function was found ($p<0.05$) in naked neck birds, which have lower T4 ($p<0.05$ - Figure 66) and higher T3 ($p<0.05$ - Figure 67) levels than in commercial broilers at the thermoneutral temperature. The naked neck birds' T4 concentrations were increased to the "normal" level (the same level as that of commercial broilers at the ambient thermoneutral temperature) and their T3 concentrations were decreased nearly to the "normal" level ($P<0.05$) by heat stress (Figures 66 and 67). Normal T4 and T3 ($P<0.05$) levels of response to TRH injection were also found in naked neck birds at 84.1 °C AET (29 °C /85 % RH) during the experiment ($p<0.05$ - Figures 68 and 69). In other words, among the three thermal load groups of naked neck birds and commercial broilers at 51.7 °C AET (29 °C 35 % RH) or 45.8 °C AET (24 °C 45 % RH), the response of T4 and T3 levels to TRH injection were the same during the experiment ($p<0.05$ - Figure 68 and 69). This result suggests that the environmental thermal load condition of 84.1 °C AET was a moderate temperature for naked neck birds whose T4 was higher than those at 24 °C and back to "normal" at 29 °C, while it depressed commercial broilers' thyroid function to hypothyroid.

Figure 65. Comparison of (1) percentage of daily food intake (%), (2) percentage of daily weight gain (% - BWG), (3) percentage of feed conversion ratio (% - FCR) of commercial broilers and naked neck chickens at 24 °C, 29 °C with low and high relative humidity (RH).

	Commercial broiler chickens			Naked neck chickens		
AET* (°C)	39.2	48.1	84.1	39.2	48.1	84.1
Room temperature	24 °C	29 °C	29 °C	24 °C	29 °C	29 °C
Relative humidity	45%RH	35%RH	85%RH	45%RH	35%RH	85%RH
Absolute humidity (gm^{-3})	8.3	10.1	24.4	8.3	10.1	24.4
Food intake	100 a (112±5.8)	98 a (117±4.6)	84 b (94±5)	100 a (79.6±8)	93 a (74±2.5)	94 a (74.9±3)
BWG	100 a (57±8)	99 a (57±2.5)	71 b (40.4±1)	100 a (32.8±0.7)	99 a (32.6±5.4)	93 a (30.5±1.5)
FCR	100 a (2.07±0.2)	100 a (2.06±0.04)	112 b (2.32±0.09)	100 a (2.43±0.2)	93 a (2.26±0.22)	101 a (2.46±0.27)

* AET = apparent equivalent temperature.

Percentage of daily values are presented as the percentage of control birds at 21 °C for birds at 29 °C with low and high relative humidity (RH) (N=6).

Values within line with different letters are significantly different at $P<0.05$ level.

A higher value for feed conversion ratio is an index of poor feed conversion efficiency.

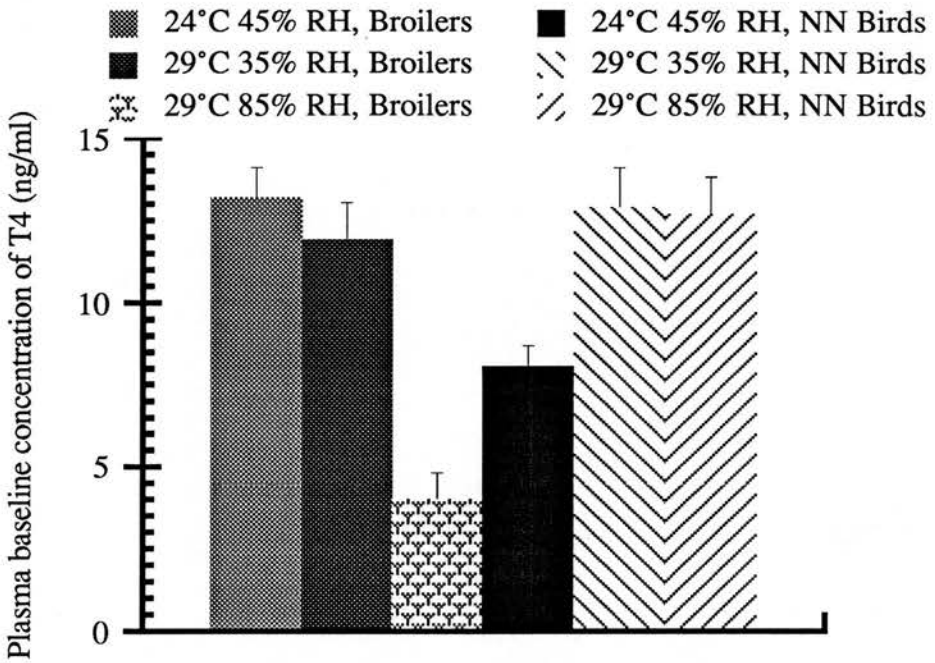


Figure 66. The effects of different thermal loads upon the baseline plasma T4 concentration (ng/ml) between naked neck (NN) birds and modern broilers. Values are expressed as means ± SEM for six female broiler chickens.

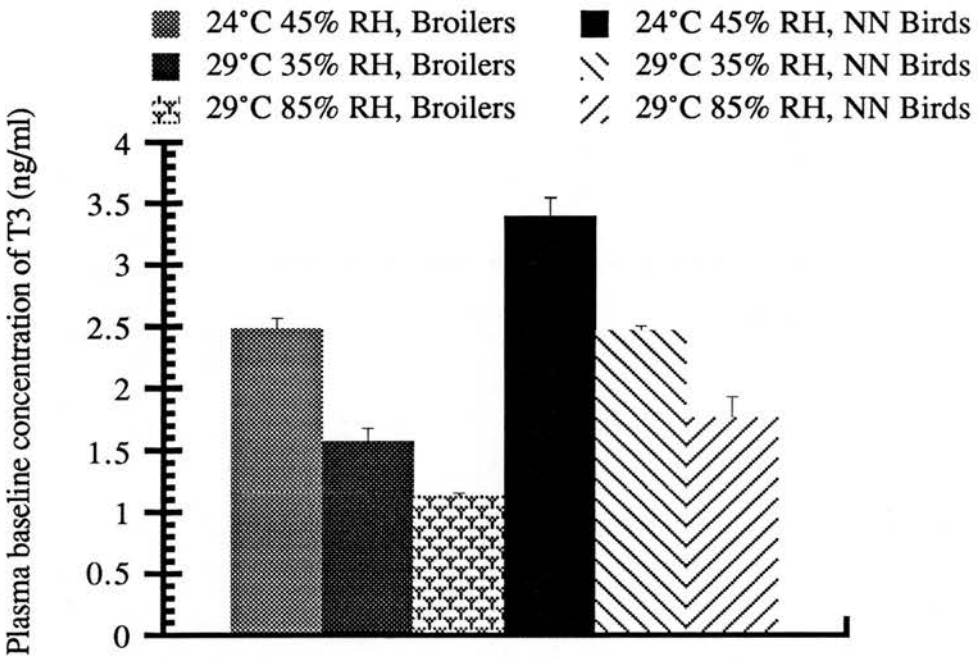


Figure 67. The effects of different thermal loads upon the baseline plasma T3 concentration (ng/ml) between naked neck (NN) birds and modern broilers. Values are expressed as means ± SEM for six female broiler chickens.

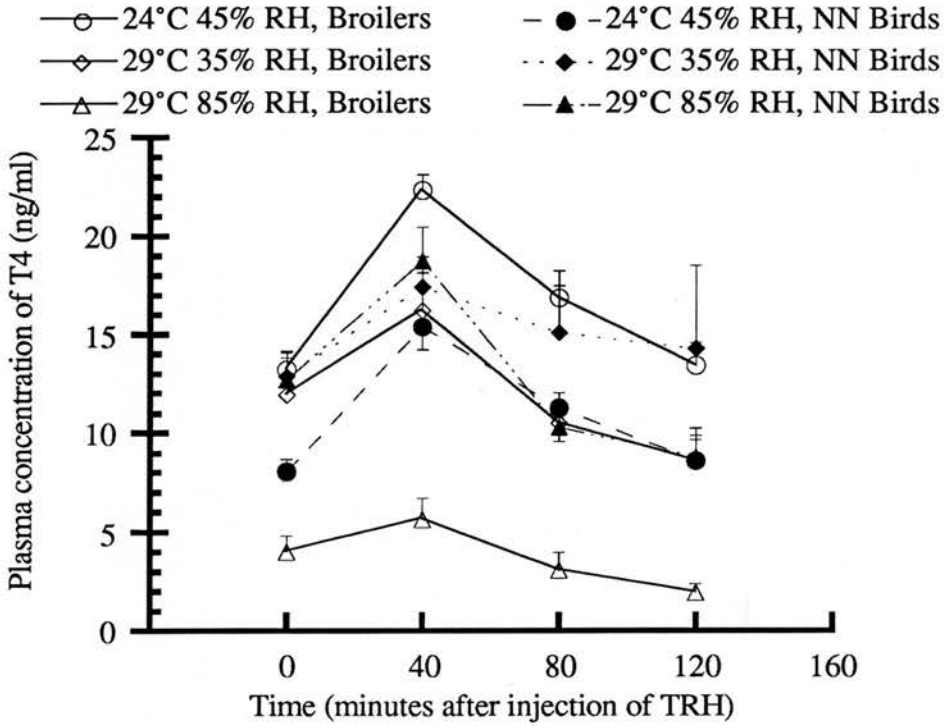


Figure 68. The effects of different thermal loads upon plasma T4 responses to the injection of TRH between naked neck (NN) birds and modern broilers. Values are expressed as means ± SEM for six female broiler chickens.

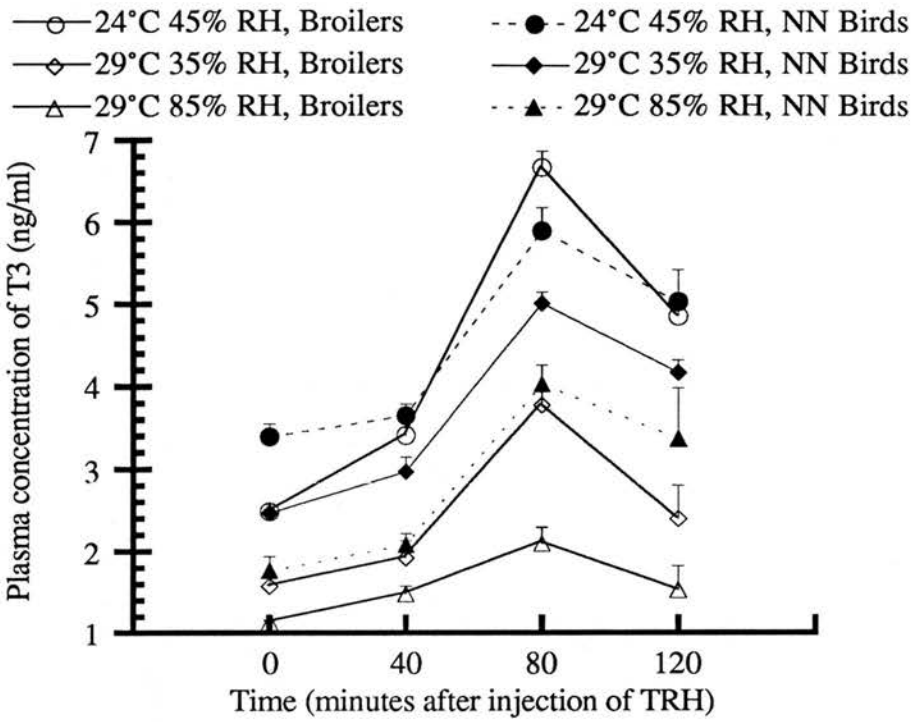


Figure 69. The effects of different thermal loads upon plasma T3 responses to the injection of TRH between naked neck (NN) birds and modern broilers. Values are expressed as means ± SEM for six female broiler chickens.

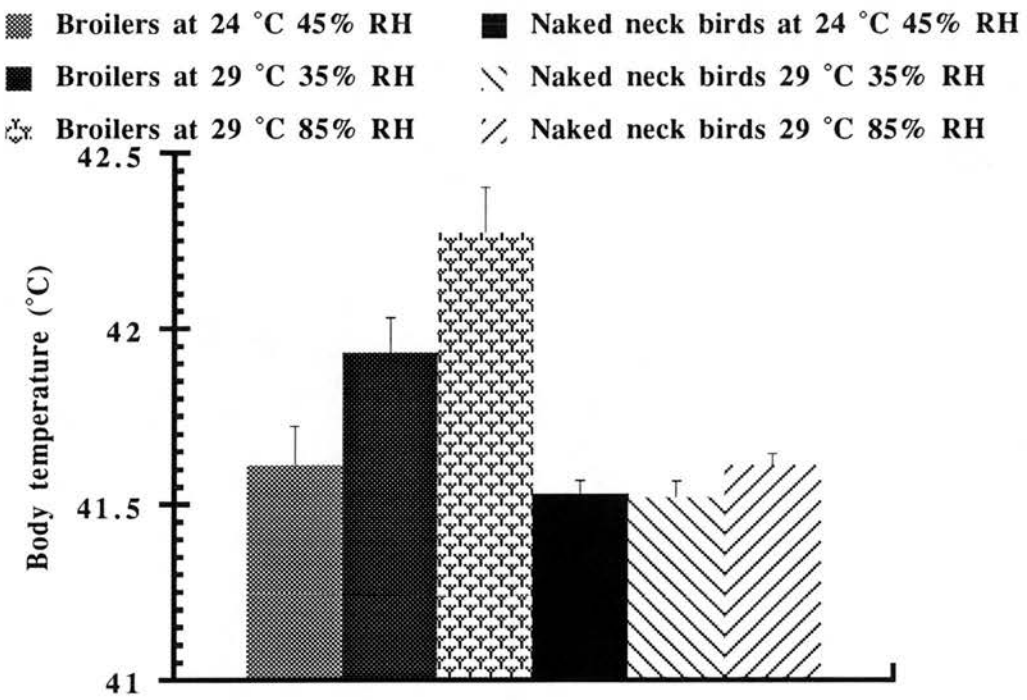


Figure 70. The effects of different thermal loads upon body temperatures between naked neck chickens and modern broilers. Values are expressed as means ± SEM for six female broiler chickens.

Rectal temperatures, used as a measure of response, were all within the normal range under "heat stress" treatment in the naked neck chickens while for the same treatment the commercial modern broiler chickens showed a significant rise of rectal temperature ($p<0.001$ - Figure 70).

6.3. Discussion

The present studies indicates that the naked neck birds have higher thyroid function at 24 °C, and it can be brought back to "normal" by heat stress. The "normal" thyroid hormone levels of the naked neck birds at high ambient temperatures should be considered as an important additional factor to predict consequences of selection for heat stress resistant birds because the thyroid hormone levels play an important role in control of heat production, growth rate, thermoregulation, plumage and molt in hens, and tolerance of heat stress.

A naked neck bird with reduced feather covering might achieve better growth performance and still be economical in hot climate poultry production, although better growth rates were not found in these studies. The relatively unaffected growth rate at heat stress treatment in the naked neck birds may be caused by small number of experimental birds, or they may be able to regard these heat loads as warm or thermoneutral conditions.

In young growing birds, those with smaller body weights generally survive longer than those with larger weights. Genetic differences in body size and its effect may be confounded with other genetic differences in resistance to heat stress. In a number of the studies which demonstrated breed differences in resistance to heat stress in adult birds, the breeds showing the most resistance to heat stress were also smaller in body weight. It was shown that a nonselected randombred population was much more resistant to heat stress than were broiler populations, the weight of the

randombred population was approximately half that of the broiler population. This association resulted in a highly significant phenotypic correlation of -0.67 between body weight and time to heat prostration (Washburn *et al.*, 1980, 1992).

The findings clearly show a greater thyroid hormonal basis may associate with their greater resistance to heat stress for the breeds with smaller body size in comparison with heavy breeds because T3 is the active thyroid functional hormone regulating heat production in birds. Fox (1980) demonstrated that feeding of 0.1% thiouracil in the diet for 4 weeks prior to heat stress increased heat stress survival time (HSST), and injection of DL-thyroxine for 3 days prior to testing significantly reduced HSST in both breeds with smaller body size and the heavy breeds. However. the effect of DL-thyroxine on reduction of HSST was greater in the heavy breeds than in the breeds with smaller body size. His results and those of Huston *et al.* (1962a) and ours suggest that the differences observed in HSST between these two breeds may be largely a reflection of differences in metabolic rate. There is some evidence that genetic variation in thyroid activity may be related to genetic variation in adaptation to heat stress. Although a number of factors such as differences in body size could be confounded with the response to heat stress, the study of Wilson *et al.* (1975), using day-old chicks which would have little differences in body weight, support a superior heat tolerance of the breeds with smaller body size.

The differences in response to heat stress observed between the breeds with smaller body size and other breeds may be related to differences in thyroid activity. The oxygen consumption of the breeds with smaller body size was significantly higher than that of the heavy breeds (Wilson *et al.*, 1975; Bobek *et al.*, 1977; Bobek *et al.*, 1980; Bohren *et al.*, 1982a,b). This is indicative of a higher basal metabolic rate (BMR) and more thyroid activity (either increased output or better utilization). In all breeds and in both sexes, O_2 consumption was greater for groups maintained in a

cooler variable environment that for groups maintained at a hotter, constant temperature. However, the O_2 consumption of the breeds with smaller body size in the high temperature was decreased more compared to those maintained in the cool environment than was that of the heavy breeds. This suggests that when exposed to high temperature, the breed with smaller body size is able to reduce its BMR (which should result in a decrease in internal heat production) to a greater degree than the bigger breed. Since thyroxine has a major influence on BMR, this would indicate a greater reduction in thyroid activity.

The findings also suggested that not only the breeds with smaller body size should be characterised in terms of their potential influence upon thermal exchange and thermoregulation but single genes which affect the feather structure or the amount of feather covering may also affect the response to environmental temperature. These include such genes as the scaleless gene (sc), the naked neck gene (Na), the frizzling gene (F), and the sex-linked slow-feathering gene ($_ZK$). Some of these genes may have merit in a selection program to shape a commercial broiler to fit a hot environment. Recently, the improving commercial modern broilers grown at high environment temperature (30-33 °C) by the Na gene has been found (Cahaner *et al.*, 1992, 1993). The improving the performance of the sex-linked silve-feathering genotype ($_ZS_ZS$) broiler males by the Na gene in the warmer environment (30 °C) has also been found although body weight was reduced by $_ZS$ feathering gene, while the Na had no effect (Lou *et al.*, 1992).

The naked neck gene, is a genetic mutant with approximately 30-40% reduced feather covering (Mérat, 1986). The genetic approach to improving performance of heat stressed birds may benefit from the use of the naked neck gene that alters the extent of feathering. Due to reduced feathering up to 30%-40%, the naked neck chicken is expected a 9%-12% better growth rate, a 6%-9% better egg weight and to

consume more food in high environmental temperature (Mérat, 1980; Mérat, 1986; Horst, 1988; Cahaner *et al.*, 1992, 1993; Lou *et al.*, 1992), and have been found to have flexibility in thermoregulation and a consistently greater survival rate to heat stress (Touchburn *et al.*, 1972). When grown at 31 °C, the Na/Na cockerels gained more from 2 to 10 weeks of age than na/na with the Na/na intermediate (Bordas *et al.*, 1978). A similar difference was not observed in birds grown under 29 °C 85% RH in this experiment.

The results and the study of Bohren *et al.* (1982a,b) points out that the importance of acclimatization to hot temperatures on response to heat stress. In their studies, lines selected for fast or slow growth in a hot (32.2 °C) and in a cold (21.1 °C) environment were reared in a hot and a cold environment from 5 to 9 weeks of age, and then exposed to a high-temperature stress of 40.6 °C. Birds reared in the cold environment consistently had higher mortality (69%) than those reared in the hot environment (21%) when subjected to the heat stress. Both of the lines selected in the hot and the cold environment responded similarly. In our studies, Na with slow growth either in a hot (29 °C with 35 or 85% RH) or in a thermoneutral (24 °C 45% RH) environment consistently had higher T3 than those commercial broilers reared in the same environment.

The present study of an inhibitory effect of heat stress (29 °C 85-95% RH) on thyroid function and the 5'-monodeiodination in commercial broilers only, but not in naked neck birds, confirmed our previous finding that the naked neck chicken may respond to the same heat load (29 °C with 85%) as moderate heat load treatment rather than severe heat load condition as those commercial broilers reared in the same environment.

This result has shown the sensitivity of the peripheral 5'-monodeiodination of T4 to T3 in the liver to administration of exogenous TRH is decreased in only the

commercial but not naked neck chickens. This result suggests that chronic high ambient temperatures and naked neck gene may play an important role in control of GH-receptor binding in chicken liver membranes and thyroid function. The future research will look for the answer why the 5'-monodeiodinase (5'-D) activity in liver cells is different among heat-stressed and non-heat-stressed commercial and naked neck chickens.

The findings and the results in the existing literature suggested that the naked neck gene could be beneficially incorporated into a commercial broiler population because the naked neck gene does not have the disadvantages of the genes which cause removal of all body feathers, and then selection pressure placed on multigenic variation in heat resistance, higher thyroid function and large body size.

There is some evidence that genetic variation in thyroid activity may be related to genetic variation in adaptation to heat stress. Bowen *et al.* (1982) compared differences in heat tolerance of Japanese quail lines selected for growth with a nonselected control. Mean survival times were higher for the nonselected control line, compared to those lines selected for growth. In this study, two of the lines had been selected for large body size on a diet containing 0.1% thiouracil. The birds selected under the thiouracil stressor were significantly less tolerant of heat than were birds selected for growth under a normal diet. It was hypothesised that during the 55 generations of selection under the thiouracil diet, there had also been selection for compensation in thyroid activity, and when not fed the diet they were hyperthyroid. A number of studies have also demonstrated the importance of the thyroid gland in the physiological adaptation to heat stress. Bowen and Washburn (1982) studied the genetic variation in thyroxine response to thyroid stimulating hormone (TSH) and the relationship of genetic variation in TSH to heat stress survival time (HSST). Heritability estimates for T4 levels, after TSH injections, were 0.63 and 0.56, based

on the sire and dam components of variance. Chicks from the six sire families previously identified as having a high response, and the six sire families identified as having a low response to TSH and a nonselected control, were heat-stressed at 4 weeks of age. Body weights were 210, 202, and 196 g and the HSST were 192, 184, and 185 min for the high, low, and control groups, respectively. These differences were not significant.

Thus, in conclusion, not only the environments should be characterised in terms of their potential influence upon thermal exchange and thermoregulation but also the breeds should be characterised in terms of their potential differences in thermotolerances, feather condition, growth rate and basic thyroid function. To develop a selection criterion for resistance to heat stress, it is desirable to put selection pressure placed on multigenic variation in heat resistance, higher thyroid function and large body size, after the naked neck gene could be incorporated into a commercial broiler population. The naked neck chicken may respond to the same heat load (29 °C with 85%RH) as moderate heat load treatment rather than severe heat load condition as those commercial broilers reared in the same environment. Different breeds, therefore, may be able to regard the same heat load (29 °C with 85%RH) as different degrees of heat stress of either severe or moderate magnitude.

In conclusion, 1) The higher thyroid function were found in naked neck birds, which have lower T4 and higher T3 levels than in normal (commercial) broilers at the ambient thermoneutral temperature. 2) Heat stress causes increase of plasma T4 concentration and decrease of plasma T3 concentration in naked neck birds. 3) "Normal" T4 and T3 levels of response to TRH injection were also found in naked neck birds at 29 °C 85 % RH during the experiment. 4) Different breeds, therefore, may be respond to the same heat load (29 °C with 85%RH) as different degrees of heat stress of either severe or moderate magnitude. 5) The confusion in responses in

the levels of T4 and T3 in heat stressed chickens in the existing literature may be the result not only of the heat loads but also the breeds.

Chapter Seven

The thyroid hormone effects of strategies (antioxidant capacities of vitamin C and E in stress protection) for reduction or alleviation of heat stress and TRH challenge in the domestic fowl

7.1. The effects of administration of ascorbic acid (vitamin C) upon plasma thyroid hormonal levels in heat stressed chickens

7.1.1. Introduction

Environmental temperature has been shown to influence vitamin requirements. As we are aware, broiler chickens and other meat animals grow very slowly at high ambient temperature. Under conditions of heat stress, the birds reduce their activity to a minimum; they find the coolest place available and tend to remain there almost motionless; they are panting to release moisture to help cool the blood stream. The effect of high environmental temperature in decreasing food consumption is generally recognized. Thus, when the environmental temperature increases, the levels of those nutrients required in definite daily amounts, such as the vitamins, must be increased in terms of percentages of the diet. Each vitamin plays a specific role in the metabolism of the animal. Some vitamins are needed for the proper functioning of every cell of the body, others are needed only under specific conditions (see Scott, 1976 for review). In order to allow the birds to grow more rapidly at high ambient temperature, dietary

ascorbic acid (AA or vitamin C) supplementation has been examined and has been reported to improve heat resistance (Pardue *et al.*, 1985b) and growth rate (Kafri and Cherry, 1984) in chickens (see Section 1.9.1, Page 98-100 for details).

In this section the effects of dietary ascorbic acid supplementation and different heat loads upon the endocrine responses of plasma concentrations of thyroxine (T4), tri-iodothyronine (T3) in broiler chickens have been investigated in order to establish the thyroid hormonal responses of the domestic fowl exposed to humid heat stress conditions after dietary ascorbic acid (AA) supplementation.

To develop a satisfactory diet for resistance to heat stress, it is desirable to understand the thyroid hormonal basis of the influence of dietary ascorbic acid on response to chronic heat stress. The objective of this study was to determine the effect of high concentrations (500 mg/kg diet) of AA on plasma T4 and T3 levels in female broiler chickens under chronic heat load conditions. Although thyroid weight showed a significant increase in layer chickens given supplementary AA (vitamin C) for 6 months (al-Janabi *et al.*, 1988), no earlier studies report plasma thyroid hormone levels of broiler chickens given supplemental AA. It was therefore considered appropriate to determine the effect of acute heat stress following by dietary high concentrations (500 mg/kg diet) of AA supplementation upon the endocrine responses of plasma concentrations of T4 and T3 in female broiler chickens under chronic heat load conditions. In addition, to study the effects of chronic different heat loads, which were produced by controlling water vapour density at either 10.1 or 24.4 gm^{-3} at a single elevated dry bulb temperature (29 °C) corresponding to RH values of 35 and 85%, upon plasma thyroid hormon levels between 3 and 6 weeks of age.

7.1.2. Experimental procedure

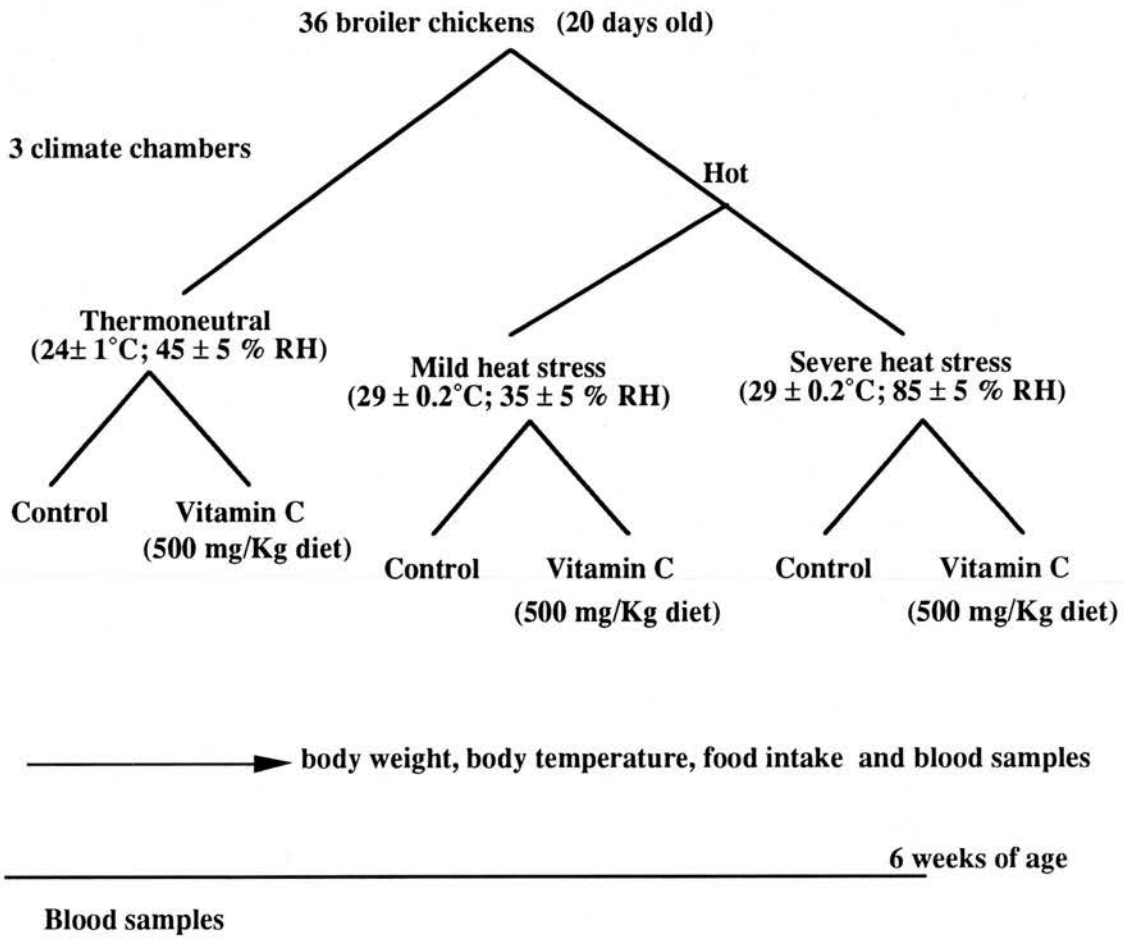
Commercial female broiler chickens (Ross Poultry (GB.) Ltd) between age 3 to 6 weeks were used in the experiment. The trial was conducted using three environmentally controlled chambers (see Figure 71 for details). The broiler chickens at age of 20 days were randomly assigned to six groups of six each (N=6). The six groups were randomly assigned to three climate chambers of 12 birds each. From 3 to 6 weeks of age, the six groups were grown under thermoneutral (L, 24 ± 1°C; 45 ± 5 % RH), moderate (M, 29 ± 0.2°C; 35 ± 5 % RH), or severe (H, 29 ± 0.2°C; 85 ± 5 % RH) heat load conditions and fed either with AA (500 mg/kg diet) or without AA from 3 to 6 weeks of age. Then, they were maintained on 23 hr light/day until 6-week of age. Food and water were provided *ad libitum*. The birds' body temperatures were measured every two days using an electronic rectal probe. Body weights were measured every two days as well. Food intakes were measured daily and growth rate and feed conversion ratio were calculated. Temperature and relative humidity in each room were controlled by the addition of conditioned air and ventilation. The actual temperature varied by no more than 1 °C around the set point.

At the end of the experimental period, plasma samples were obtained at 11:00 am from 6 birds by venipuncture (brachial vein) in each treatment. Blood plasma was prepared by centrifugation at 1500 *g* for 10 minutes and stored at -20°C prior to T4 and T3 radioimmunoassay (RIA). Plasma concentrations of thyroxine (T4) and tri-iodothyronine (T3) were determined by radioimmunoassay (see Section 2.3.1, Page 114 for details).

7.1.3. Results

The comparisons of the effect of acute heat stress following by chronic different heat stress conditions upon plasma thyroid hormone concentrations in

Figure 71. Diagram of experimental procedure



growing broiler chickens received diets with or without supplementary AA (vitamin C) are summarised in figures 72-76.

As expected, the chronic heat stress treatments had pronounced effect on food intake, feed conversion ratio and body weight gain (Figure 72). Broiler chickens under heat stress showed significant reduction of body weight gain ($p<0.05$ - Figure 72). Supplementary ascorbic acid (AA or vitamin C) seems to have no effect on these parameters.

Higher thyroid function, which gave higher plasma T3 concentration ($p<0.02$ - Figure 73) was found in chickens given supplementary vitamin C at the severe heat stress condition for 21 days than those without supplementary vitamin C. The results indicated that supplemental vitamin C can be effective in increasing plasma T3 levels of chickens under severe heat stress. However, no differences of the plasma T3 concentrations were found between the broilers fed with and without vitamin C under environmental thermoneutral conditions or the moderate heat stressed chickens.

Although the above parameter was improved ($P<0.02$) due to feeding vitamin C in the chronic severe heat stressed growing chickens, the plasma T3 concentrations in the chronic heat stressed chickens given supplemental vitamin C were still profoundly depressed and were 35% lower ($P<0.001$) than that in thermoneutral conditions.

Chickens given supplementary vitamin C at all heat stress condition for 21 days, however, did not improve their plasma T4 concentration in comparison with those without supplementary vitamin C, despite decreased absolute concentrations at severe heat stress condition ($p<0.05$ - Figure 74).

The result also show that heat stress made the moderately heat stressed birds increase their blood pH value, and their increased blood alkalosis can be returned to

Figure 72. Comparison of (1) percentage of daily food intake (%), (2) percentage of daily weight gain (% - BWG), (3) percentage of feed conversion ratio (% - FCR) of broiler chickens administration with ascorbic acid (AA or vitamin C) (500 mg AA /kg diet) at 24 °C, 29 °C with low and high relative humidity (RH).

	Thermoneutral		Moderate heat stress		Severe heat stress	
	Control	AA	Control	AA	Control	AA
AET* (°C)	45.8	45.8	51.7	51.7	84.1	84.1
Room temperature	24 °C	24 °C	29 °C	29 °C	29 °C	29 °C
Relative humidity	45%RH	45%RH	35%RH	35%RH	85%RH	85%RH
Absolute humidity (gm^{-3})	9.8	9.8	10.1	10.1	24.4	24.4
Food intake	100 a (121.25±2.64)	97 a (117.4±8.47)	86 b (104.42±6.197)	89 b (108.21±4.029)	71 c (85.9±5.65)	70 c (84.7±26.167)
Body weight gain	100 abc (52.26±23.271)	104 a (54.1±2.627)	83 b (43.5±3.989)	98 abc (51±20.772)	73 c (38.33±4.31)	68 c (35.6±4.771)
FCR	100 ab (2.32±1.941)	94 a (2.17±0.16)	103 ab (2.40±0.23)	91 ab (2.12±1.138)	109 b (2.54±0.409)	103 ab (2.38±0.936)
FI (g/day)	100 a	97 a	86 b	89 b	71 c	70 c
BWG (g/day)	100 abc	104 a	83 b	98 abc	73 c	68 c
FCR (FI/BWG)	100 ab	94 a	103 ab	91 ab	109 b	103 ab

* AET = apparent equivalent temperature.

Percentage of daily values are presented as the percentage of control birds at 21 °C for birds at 29 °C with low and high relative humidity (RH) (N=6).

Values within line with different letters (superscripts) are significantly different at $p<0.05$ level.

A higher value for feed conversion ratio (FCR) is an index of poor feed conversion efficiency.

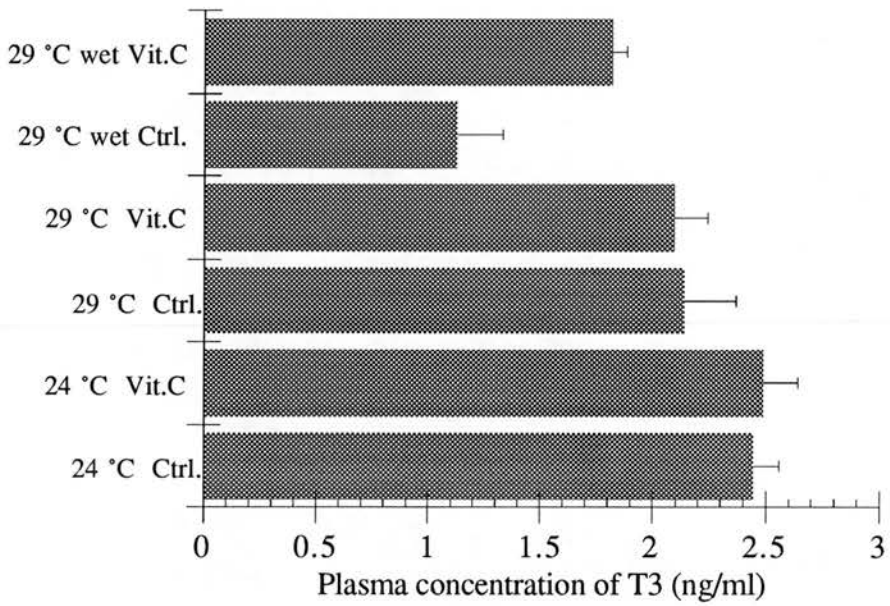


Figure 73. The effects of administration of ascorbic acid (vitamin C) upon plasma T3 concentration in modern broiler chickens exposed to different humid hyperthermal load conditions. Values are expressed as means ± SEM for six female broiler chickens.

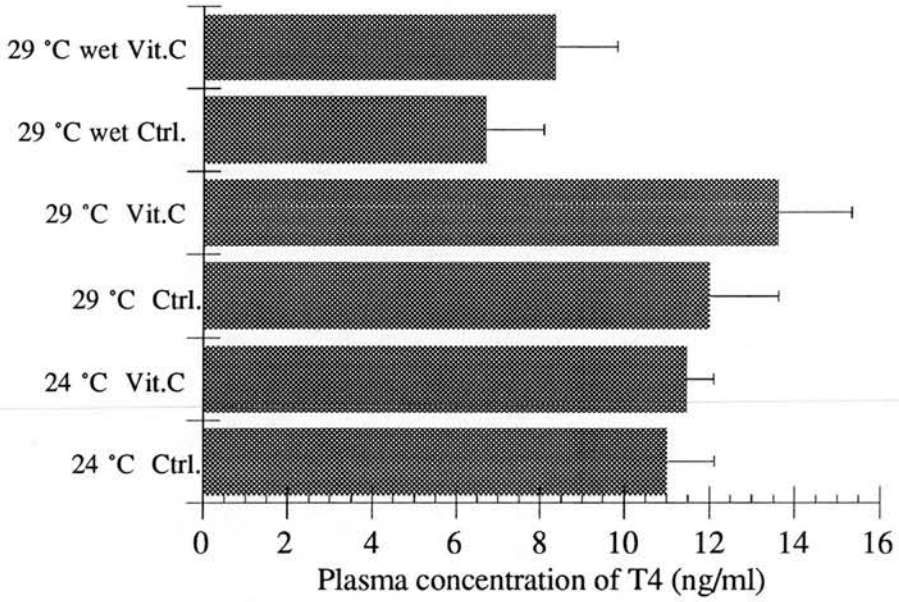


Figure 74. The effects of administration of ascorbic acid (vitamin C) upon plasma T4 concentration in modern broiler chickens exposed to different humid hyperthermal load conditions. Values are expressed as means ± SEM for six female broiler chickens.

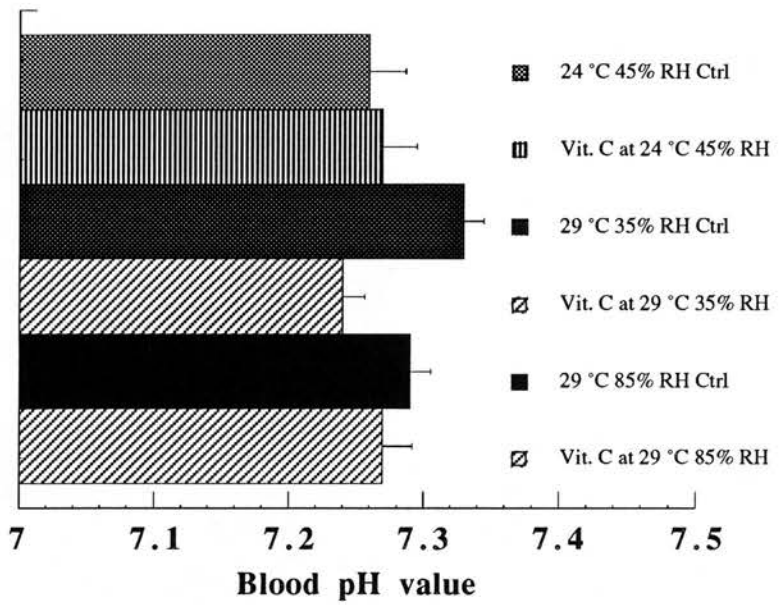


Figure 75. The effects of administration of ascorbic acid (vitamin C) upon blood alkalosis in modern broiler chickens exposed to different humid hyperthermal load conditions. Values are expressed as means ± SEM for six female broiler chickens.

Figure 76. The effects of administration of ascorbic acid (vitamin C) upon body temperature in modern broiler chickens exposed to different humid hyperthermal load conditions. Values are expressed as means ± SEM for six female broiler chickens.

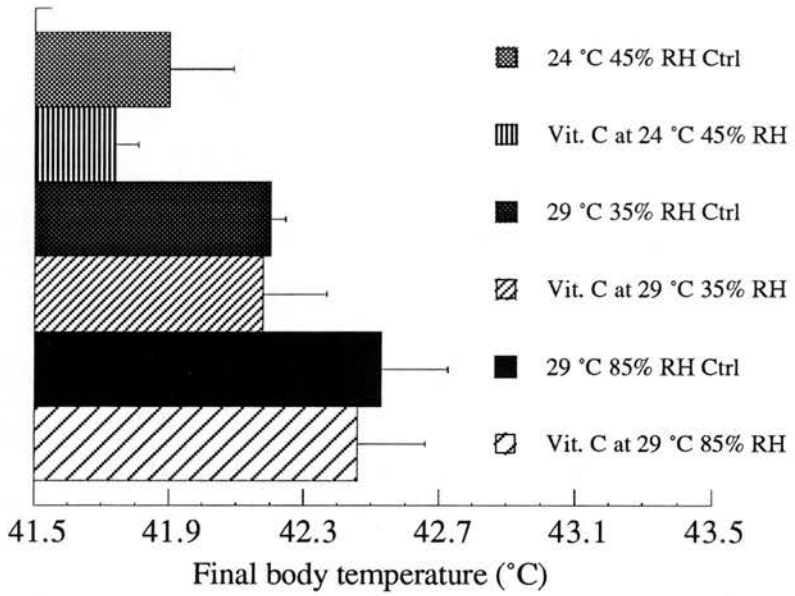


Figure 76a. The effects of different heat loads upon final body temperatures.

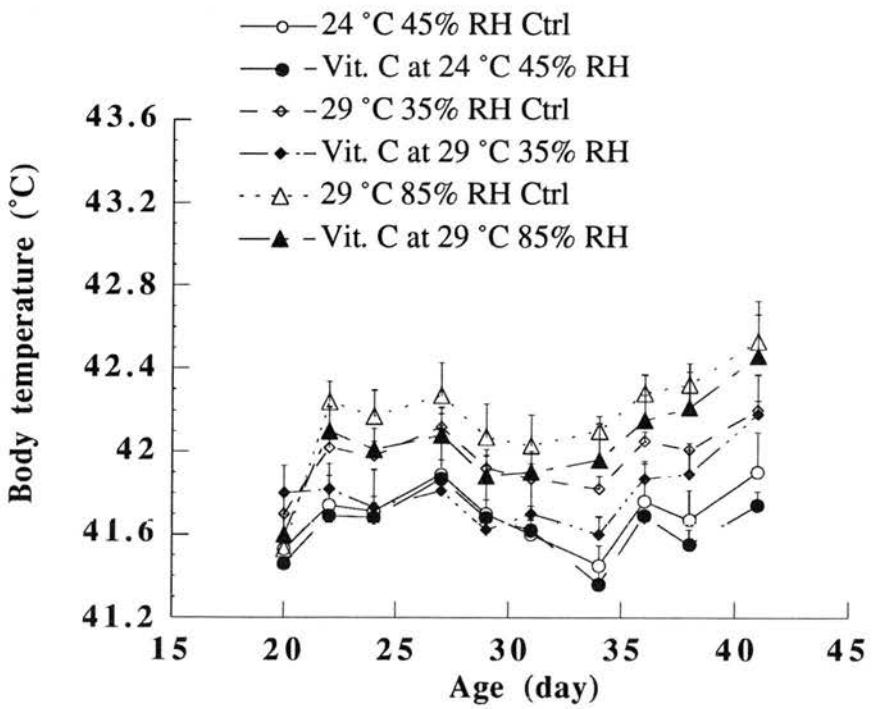


Figure 76b. The effects of different heat loads upon body temperatures throughout the whole heat stress period.

the normal level by administration with ascorbic acid ($p<0.05$ - Figure 75), although the blood pH values of the severe heat stressed birds in both with and without ascorbic acid groups were not changed.

The 21-day chronic severe and moderate heat stress treatments had no effects on feed conversion ratio and body weight gain (Figure 72) among all groups, although the body temperature (Figure 76) was gradually increased by heat stress proportionally by response to heat load. Chickens given supplementary vitamin C at all heat stress condition for 21 days, however, did not improve their body temperature (Figure 76) and food intake (Figure 72) in comparison with those without supplementary AA.

7.2. The effects of administration of vitamin E (α-tocopherol) upon plasma thyroid hormone levels in heat stressed chickens

7.2.1. Introduction

The results from Section 7.1 suggested that ascorbic acid may use its reducing agent biochemical properties to prevent oxidation as a preservative in the severe heat stress condition and use its chemical acidity properties to reduce alkalinity as a stabiliser in the moderate heat stress condition. However, which biochemical properties of AA did improve (reduce the impairment of) the plasma thyroid hormone levels in heat stress broilers, still remain mystery.

From the previous result of which the administration of AA to broilers appears to reduce the impairment of the plasma T3 levels while appears not to improve (reduce the impairment of) the blood pH values in the severe heat stressed chickens, it may be interpreted as AA used its reducing agent biochemical properties to prevent oxidation in order to improve the plasma thyroid hormone levels due to a heat stress condition challenge in chronic heat stress condition reared chicks.

If the previous proposition is correct, administration of vitamin E to broilers may appear to improve the plasma thyroid hormone levels while appears not to improve the blood pH values in the severe heat stressed chickens as well, because the effects of ascorbic acid supplementation on the tissue antioxidant status seem to be dependent on the dose of ascorbic acid and the vitamin E status. When the animals are marginally adequate in vitamin E status, ascorbic acid supplementation in large doses appears to promote lipid peroxidation and significantly decreases the antioxidant potential of animals (see Chen 1992 for review). The effects of administration of vitamin E to broilers may also influence by difference heat load conditions as administration of ascorbic acid.

In this section the effects of dietary vitamin E supplementation and different heat loads upon the endocrine responses of plasma concentrations of thyroxine (T4) and tri-iodothyronine (T3) in broiler chickens have been investigated in order to establish the thyroid hormonal responses of the domestic fowl exposed to humid heat stress conditions after dietary vitamin E supplementation.

To develop a satisfactory diet for resistance to heat stress, it is desirable to understand the thyroid hormonal basis of the influence of dietary vitamin E supplementation on response to chronic heat stress. The effects of dietary vitamin E supplementation on plasma thyroid hormone levels and their performance in heat stressed broilers, however, have not been studied. In order to allow us to predict whether the potential influence upon preventing oxidation of ascorbic acid using its reducing agent biochemical property as a preservative could reflect its activities as a regulator of plasma thyroid hormone levels in the severe heat stress condition, we carried out this experiment to investigate if an increase of the level of vitamin E supplementation could improve plasma thyroid hormone levels in heat stressed broilers. In addition, the objective of the study was also to determine the effect of high

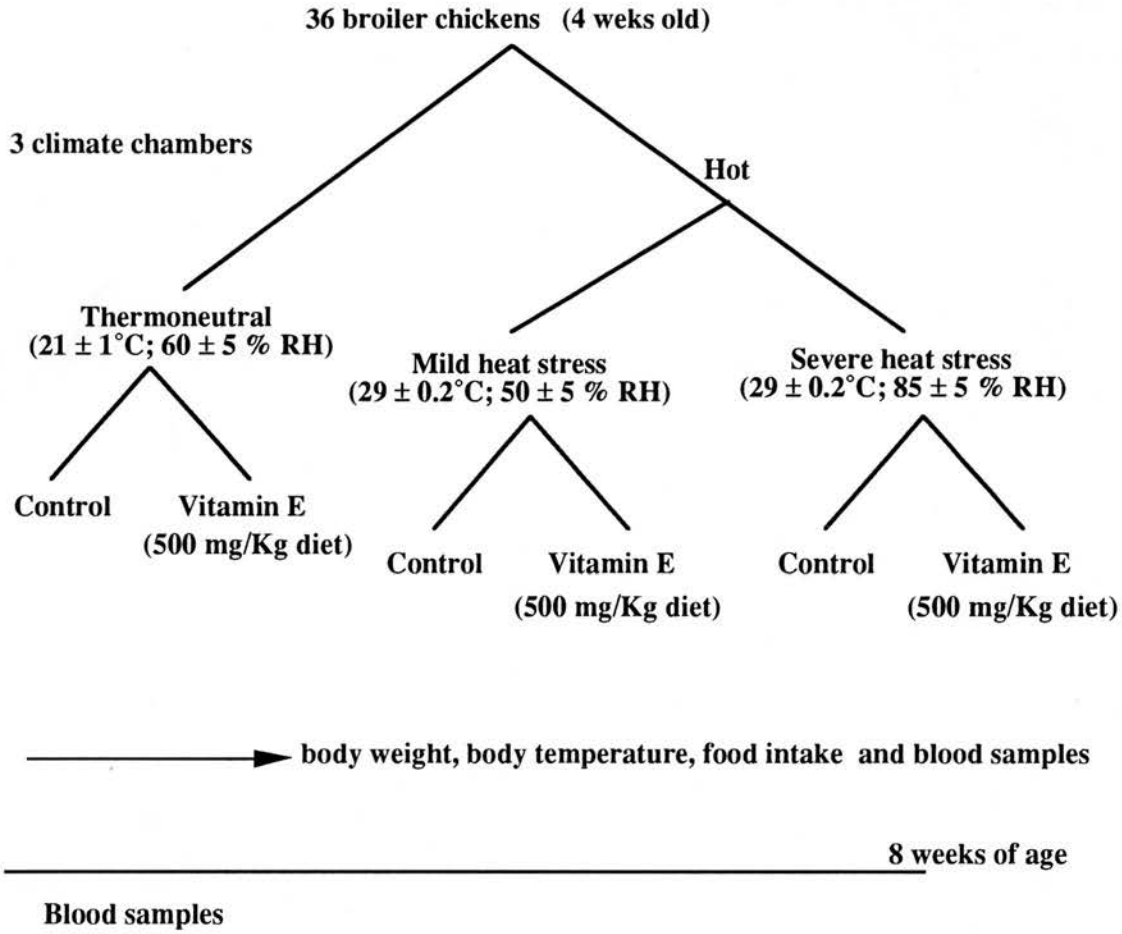
levels of dietary vitamin E on the performance of broiler chickens under different chronic heat load conditions achieved by maintaining broilers at a single ambient temperature (29 °C) with two humidities (50% or 85% RH) and feeding them with or without vitamin E. Control birds were maintained at 21 °C and 60 % RH and feeding them with or without vitamin E as well. In the present chapter, we report the results of such studies.

7.2.2. Experimental procedure

36 broiler chickens (four-week old) were employed in this study. These birds had been reared at a temperature of 21 °C from 3-4 weeks of age. They were divided into three climate rooms at a single ambient temperature (29 °C) with two humidities (50% or 85% RH) and control birds were maintained at 21 °C and 60 % RH (see Figure 77 for details). The three groups were further subdivided into two sub groups in each of the three climate rooms where two modules were used, facing each other. Half of each group received a standard broiler diet as a control diet and the other half received the same diet supplemented with 500 mg/kg vitamin E as a vitamin E supplemented diet. The birds were put in the cages in such a way that the birds on the control diet were facing those on the vitamin E diet. Six birds were exposed to each treatment for 21 days from 4 to 8 weeks of age.

The birds were given food and water *ad libitum* and were fed each morning. The food put in and the food put out were recorded every morning, the difference was calculated which gave the food eaten every day by the individual birds. The performance of broiler chickens under chronic heat load conditions has also been determined.

Figure 77. Diagram of experimental procedure



The room temperature and relative humidity were measured every morning using the wet and dry bulb thermometer, a whirling hygrometer was also used to determine the relative humidity.

The birds' body weights and temperature were measured before putting the birds into the climate rooms. The birds' body temperatures were measured every two days using an electronic rectal probe. The body weights were measured every 5 days.

Blood samples (2 mls) were always obtained at 11:00 am from 6 birds by venipuncture (brachial vein) in each treatment every time during this exposure period. The samples were divided into two aliquots. One was placed in heparinised tubes which were placed in ice as they were being taken for the blood gas analysis. The heparinised blood samples were analysed for blood pH and gases using a Corning model 238 pH/blood gas analyser (Ciba-Corning). The second aliquot was put in EDTA tubes for subsequent haematological analyses. Blood plasma was prepared from the heparinised samples by centrifugation at 1500 g for 10 minutes and stored at -20 °C pending analysis. Plasma concentrations of thyroxine (T4) and tri-iodothyronine (T3) were determined by radioimmunoassay (see Section 2.3.1, Page 114 for details).

7.2.3. Results

The comparisons of the plasma thyroid hormone concentrations during chronic different heat stress conditions in commercial broiler chickens given diet with or without supplementary vitamin E are summarised in figures 78-81.

There was a slight increase ($P>0.05$) in the body weight gain of birds on vitamin E kept at 84.1 °C AET (29 °C / 85% RH), although there was a significant difference between birds on vitamin E and control diets kept at 21 °C ($p<0.05$ - Figures 78 and 79).

Figure 78. Comparison of (1) percentage of daily food intake (%), (2) percentage of daily weight gain (% - BWG), (3) percentage of feed conversion ratio (% - FCR) of broiler chickens administration with vitamin E (Vit.E) (500 mg α-tocopherol /kg diet) at 21 °C, 29 °C with low and high relative humidity (RH).

	Thermoneutral		Moderate heat stress		Severe heat stress	
	Control	Vit.E	Control	Vit.E	Control	Vit.E
AET* (°C)	45.3	45.3	61.4	61.4	84.1	84.1
Room temperature	21 °C	21 °C	29 °C	29 °C	29 °C	29 °C
Relative humidity	60%RH	60%RH	50%RH	50%RH	85%RH	85%RH
Absolute humidity (gm^{-3})	11.0	11.0	14.4	14.4	24.4	24.4
Food intake	100 a (791±55)	87 ab (691±38)	84 bc (660±54)	75 c (596±26)	65 d (511±39)	53 d (420±52)
Body weight gain	100 ab (224±7)	113 a (252±20)	87 c (195±14)	88 abc (197±48)	65 c (145±37)	77 bc (172±28)
FCR	100 a (2.5±0.2)	87 a (2.2±0.2)	91 a (2.3±0.14)	95 a (2.34±0.17)	117 b (2.9±0.2)	96 a (2.4±0.2)

* AET = apparent equivalent temperature.

Percentage of daily values are presented as the percentage of control birds at 21 °C for birds at 29 °C with low and high relative humidity (RH) (N=6).

Values within line with different letters are significantly different at $P<0.05$ level.

A higher value for feed conversion ratio is an index of poor feed conversion efficiency.

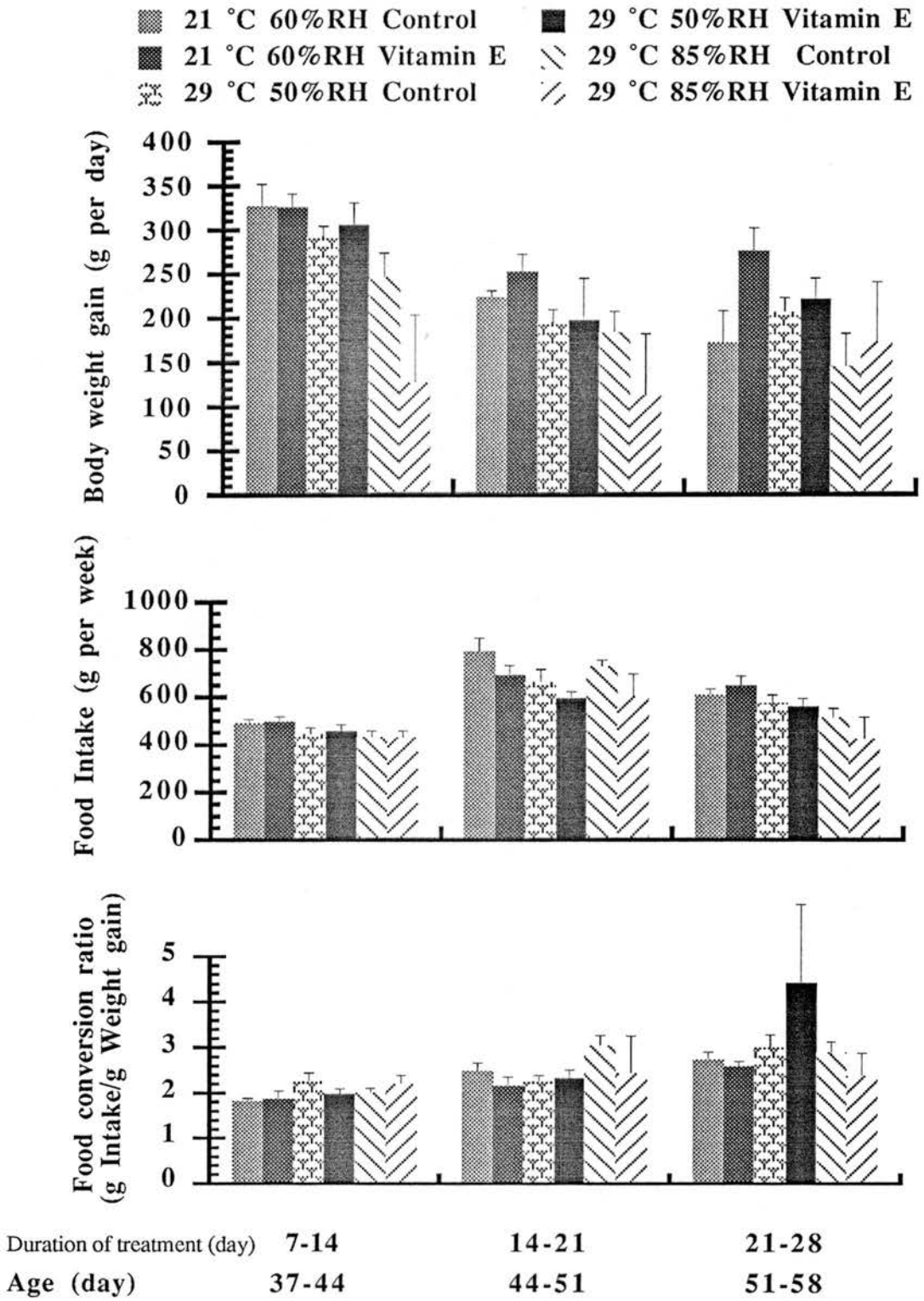


Figure 79. The effects of administration of vitamin E upon growth performances in broiler chickens exposed to different heat load conditions. Values are expressed as means ± SEM for six female broiler chickens.

Figure 80. The effects of administration of vitamin E upon plasma T4 and T3 concentration in broiler chickens exposed to different heat load conditions. Values are expressed as means ± SEM for six female broiler chickens.

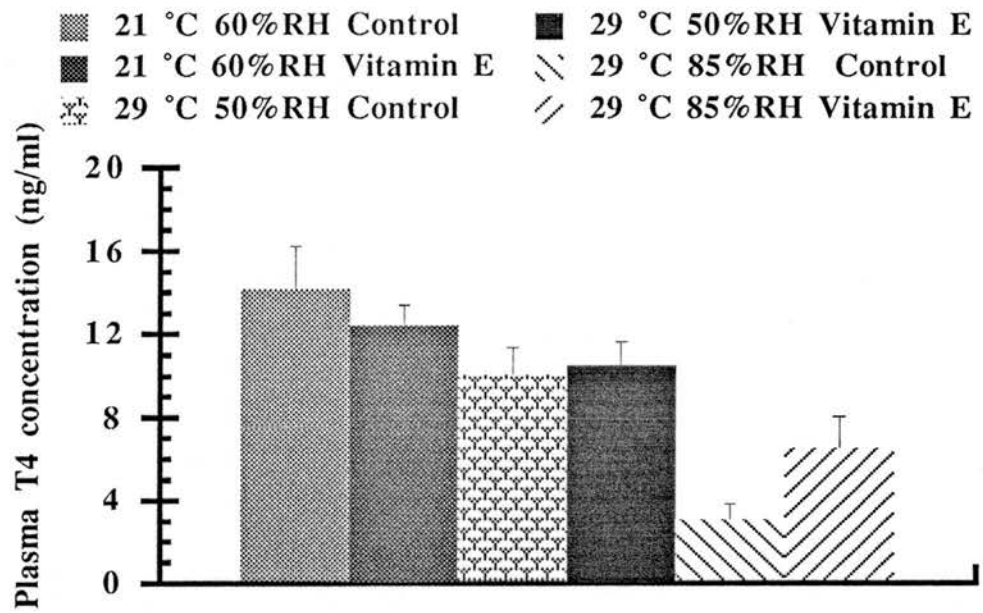


Figure 80a.

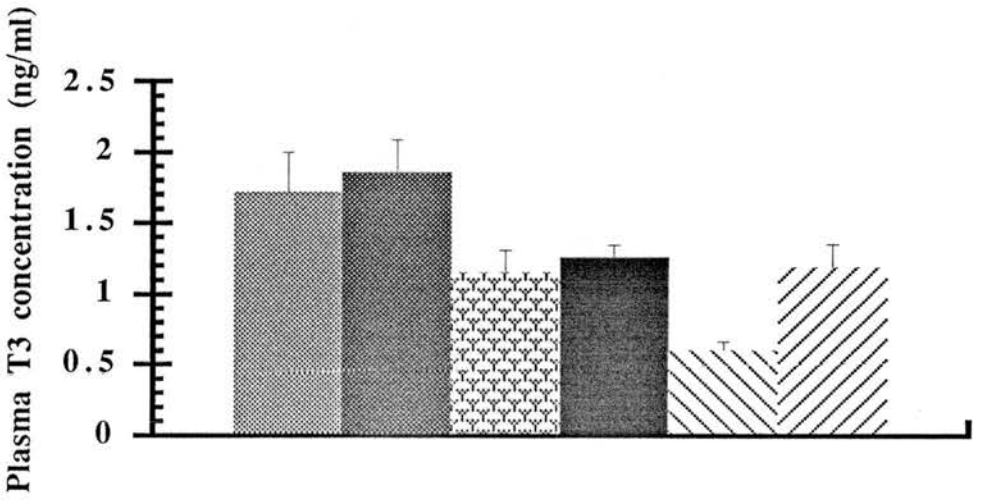
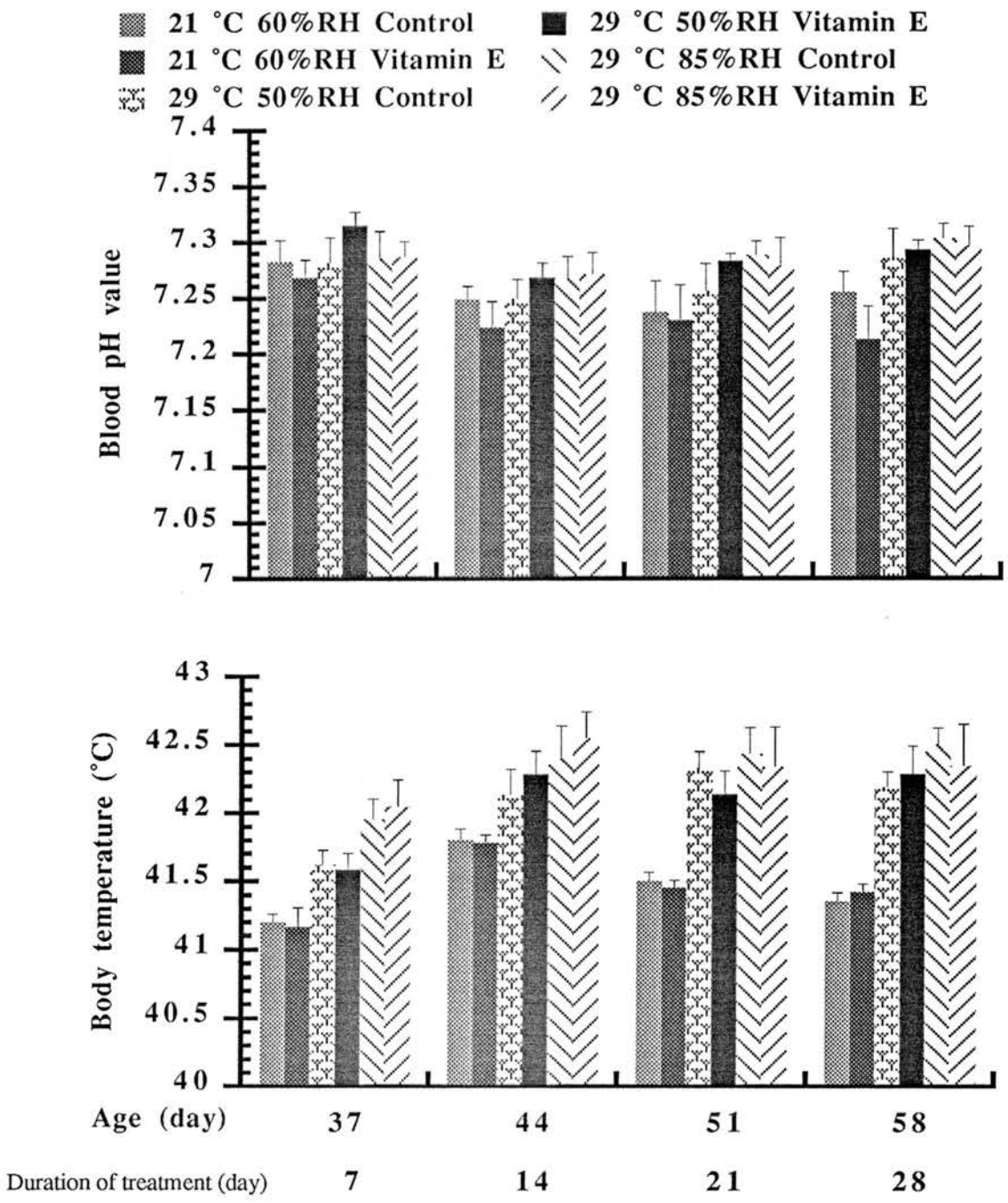


Figure 80b.

Figure 81. The effects of administration of vitamin E upon blood pH value and body temperature in broiler chickens exposed to different heat load conditions. Values are expressed as means ± SEM for six female broiler chickens.



From the data it can be suggested that the presence of vitamin E in the ration had some influence on the rate of growth when birds under stress condition. Because there was a significant difference in the body weight gain between birds on vitamin E and control diets kept at 21 °C ($p<0.05$ - Figure 79), although there was no significant difference but only slight improved (not significantly $P>0.05$) between birds on vitamin E and control diets kept at 84.1 °C AET (29 °C / 85% RH).

The food intake was decreased by heat stress proportionally to heat load. Birds at 84.1 °C AET (29 °C / 85% RH) ate less food than those in the room at 61.4 °C AET (29 °C / 50% RH) (Figure 78). However, both groups at 29 °C ate less food than those at 21 °C for both diets. A slight (not significantly $P>0.05$) decline in the feed conversion efficiency (a slight increase in the values of feed:gain ratios) was seen in birds in the high humidity group (84.1 °C AET - Figure 78).

Higher plasma T3 ($p<0.01$ - Figure 80b) and T4 (not significantly $p<0.10>0.05$ - Figure 80a) concentrations were found in chickens given supplementary vitamin E at the severe heat stress condition for 21 days than those without supplementary vitamin E. The results indicated that supplemental vitamin E can be effective in increasing plasma T3 levels of chickens due to severe high environmental temperature stress. The moderate heat load (29 RH 50 %) significantly reduced plasma T3 ($p<0.05$) but not T4. The severe heat load (29 RH 85 %) further reduced plasma T3 to significantly lower than that of moderate heat load (29 RH 50 %) ($p<0.05$) and decreased plasma T4 ($p<0.001$).

Although plasma T3 level was improved ($p<0.01$ - Figure 80a) due to vitamin E in the chronic severe heat stressed growing chickens, the concentration was still 28% lower ($P<0.05$) than that in environmental thermoneutral conditions.

The results indicated that supplemental vitamin E can slightly ($p<0.10>0.05$ - Figure 80b) increase plasma T4 levels of chickens under severe high environmental temperature stress. In chickens given supplementary vitamin E at the moderate heat load (29 RH 50 %), however, vitamin E did not improve plasma T4 concentration despite significantly reduced plasma T4 ($p<0.001$) at the moderate heat load.

There was no significant difference ($P>0.05$) in the pH level between all the birds fed with vitamin E and control diets throughout the chronic heat experiment. The result also showed that heat stress made birds increase their blood pH value. There was a significant increase ($P<0.05$) in the pH of birds at 29 °C low humidity feeding on the vitamin E diet in comparison with those kept in environmental thermoneutral conditions (21 °C 60% RH - Figure 81a), although no significant difference ($P>0.05$) in the pH level of all the birds on control diet throughout the chronic heat experiment.

In chickens given supplementary vitamin E under all heat stress condition for 27 days, however, can not improve their body temperature (Figure 81b) was not reduced in comparison with those without supplementary vitamin E. The body (rectal) temperature (Figure 81b) was increased proportionally by heat stress. Body temperature showed a significant difference ($P<0.001$) between birds at 21 °C and 29 °C high humidity on day 37 and 56 for both diets. A difference between 21 °C and 29 °C low humidity ($P<0.001$) was also recorded on 58 days of age (Figure 81b).

7.3. Discussion

The changes in body temperature (Tb) and hormonal concentrations in chronic heat stressed chickens related to ambient apparent equivalent temperatures (AET) or room temperatures (dry bulb temperatures) are summarised in figures 82-83. Each observation represents means obtained from chickens of all experiments including naked neck birds and those given ascorbic acid (AA or vitamin C) and vitamin E.

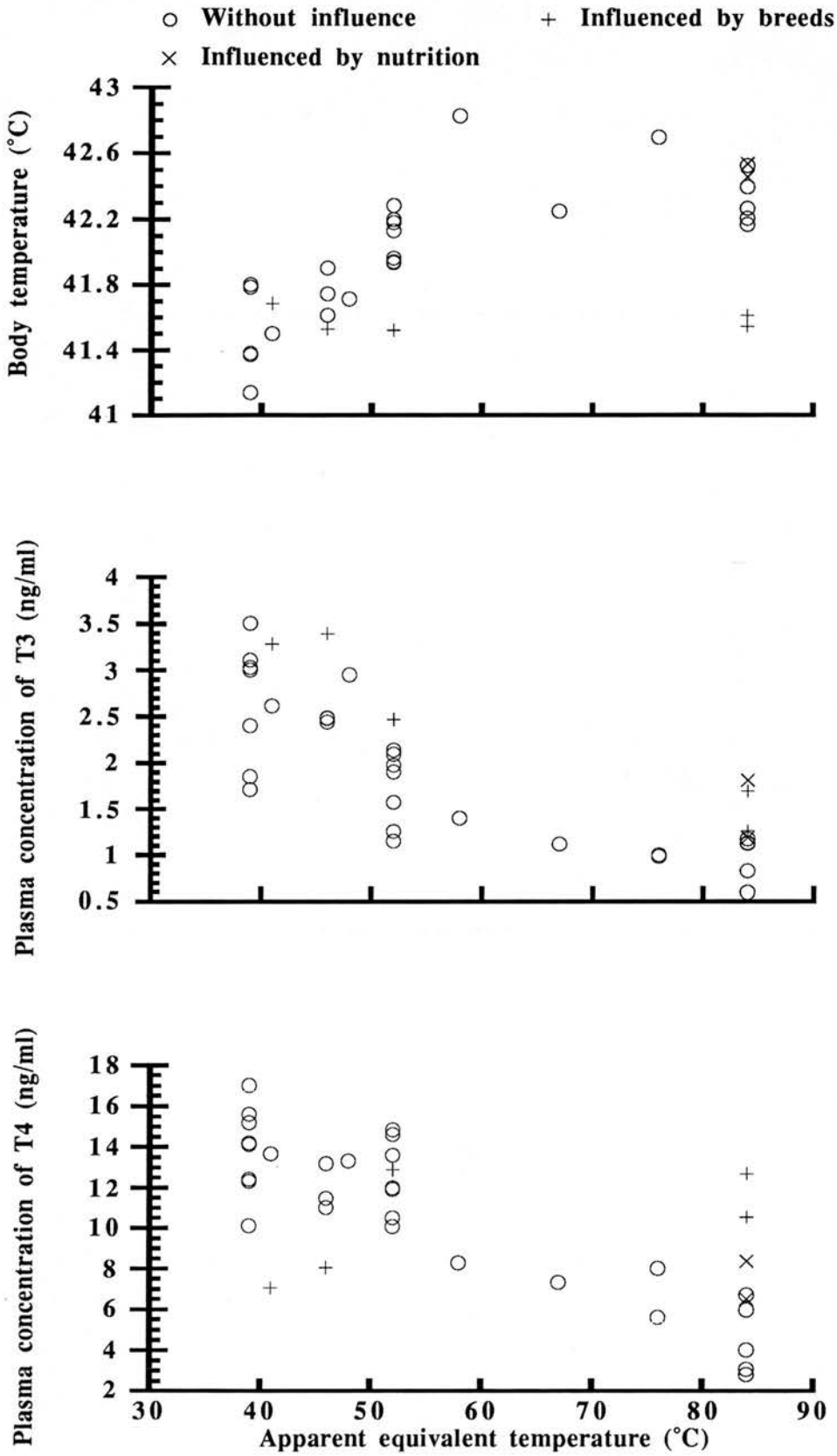


Figure 82. The influence of nutrition (dietary vitamin C and E) and breeds (naked neck birds) upon the relationship between elevated ambient apparent equivalent temperatures and changes of body temperatures (Tb) and hormonal concentrations.

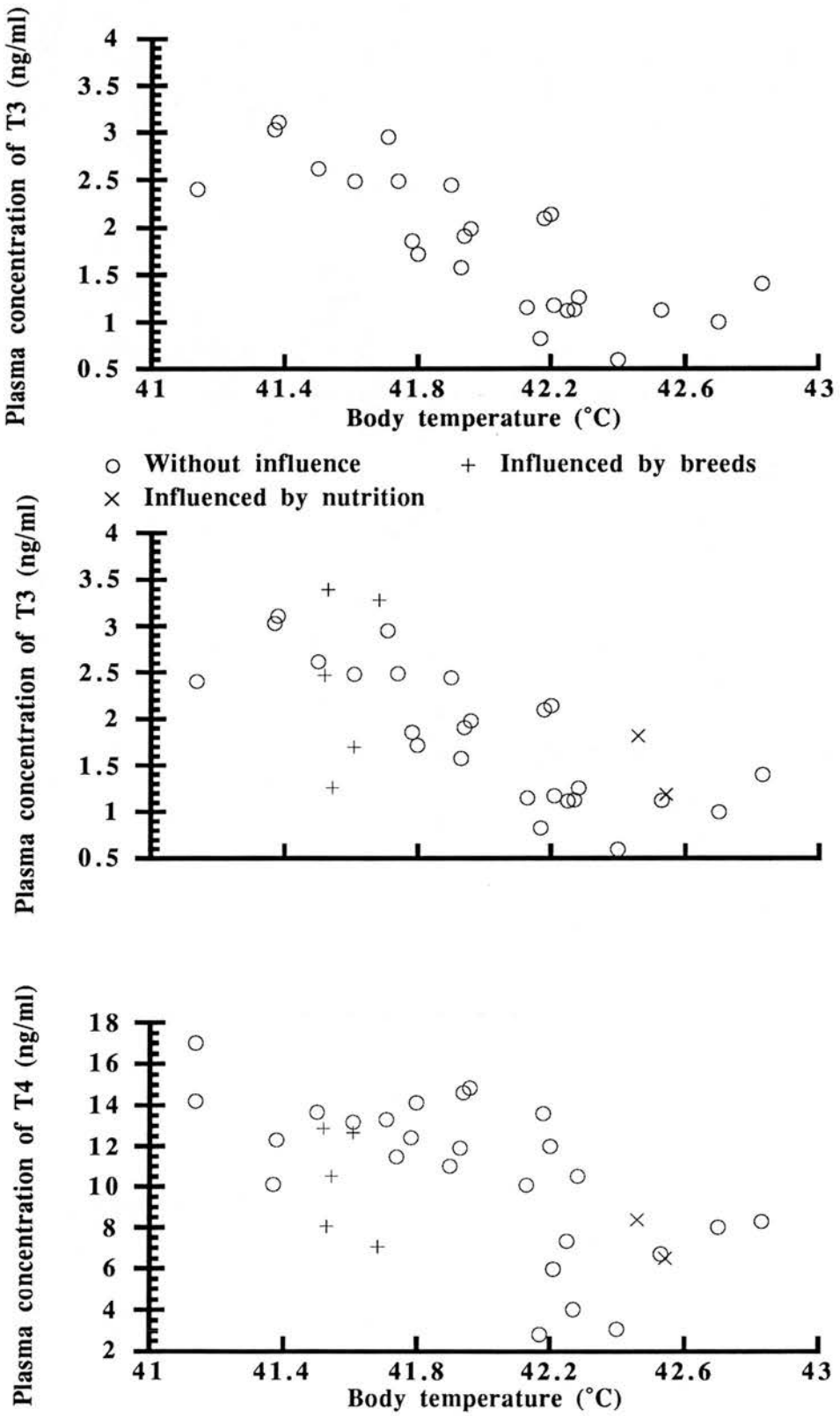


Figure 83. The relationship between changes of the peripheral T3 or T4 concentrations and body temperatures (Tb) in chronic heat stressed chickens.

The improvement of endocrine function by administration of AA and vitamin E in heat stressed chickens, might suggested that to define an environment simply by measurement of dry bulb temperature would not be enough to identify whether birds were under severe or moderate heat stress. When birds under heat stress condition, not only temperature and humid should be measured, but also breeds and nutrition status should be characterised in terms of their potential influence upon thermal exchange and thermoregulation and the endocrine response to high environmental temperatures (Figure 82). Body temperature seemed to be a good indicator of whether birds were under severe or moderate heat stress (Figure 83). Moderate heat stress could be defined as a body temperature increase from 41.9 °C to 42.3 °C (Figure 82), whilst severe heat stress could defined as a body temperature increase to 42.5 °C and above (Figure 83). The confusion in responses in the levels of T4 and T3 in heat stressed chickens in the existing literature may be resulted by not only from the environments, but also the breeds and the diets.

This current study appears to be the first of its kind concerning the effect of dietary AA or vitamin E on the plasma thyroid hormone levels in broilers. This result suggests that the supplemental dietary AA and supplementary vitamin E could improve the impairment of thyroid hormones of heat stressed broiler chickens to a more normal level, and increase the blood pH values were not accompanied by an increase in the plasma thyroid hormone levels. The administration of ascorbic acid and vitamin E to broilers appear to improve the plasma thyroid hormone levels only in the severely heat stressed chickens. The finding seem to agree with the finding of Al-Janabi *et al.* (1988) who found a significant increase ($P<0.05$) in thyroid weight and body weight after the addition of AA to layer hens kept in the summer in Iraq. As we are aware, ascorbic acid appears to have two opposite roles in animal tissues: to act as an antioxidant or to act as a prooxidant. The results suggested that supplemental dietary AA might use its reducing agent biochemical properties to prevent oxidation as a

preservative, rather than its acidity properties to improve their plasma thyroid hormone levels. The improved plasma thyroid hormone levels and the hepatic 5'-monodeiodinase activities obtained by administering AA may be associated with increased growth rate (Pardue *et al.*, 1984), thyroidal weight (Al-Janabi *et al.*, 1988) and thyroid activity in the heat stressed chickens because hyperthyroidism is associated with an increase in thyroidal weight (Ganong 1985).

The data presented here confirm the previous findings that chronic severe heat stress for periods of 3 weeks inhibits hepatic 5'-deiodination of T4 to T3 and produces significant decreases in plasma T4 and T3 concentrations. The present results showed that the suppression of the plasma T3 and T4 levels could be counter-acted by high levels of supplemental dietary AA or dietary vitamin E administration in chronic severe heat stressed birds. In addition the possible role of vitamin E (α-tocopherol) or ascorbic acid in the alleviation of such effects by way of their antioxidant properties was the possible explanation. These specific stress responses have been correlated throughout with the effects of heat stress upon growth, feed intake, feed conversion ratio, body temperatures and possible disturbances in acid-base balance or blood chemistry including pH, pCO_2, pO_2, HCO_3^- as a result of thermal panting. These parameters indicate the magnitude of the birds thermoregulatory response to a given thermal load.

From the data it might be concluded that the presence of vitamin C or E in the ration had little or no influence on the rate of growth. In deed, vitamin E improved growth and feed conversion ratio under severe heat stress and vitamin C improved growth and feed conversion ratio under moderate heat stress. It can be explained by the small number of experimental birds. Considerable evidence exists which strongly indicates that under conditions of high environmental temperatures, birds may not be able to synthesise sufficient ascorbic acid to replace the severe losses of this vitamin,

although poultry under normal environmental conditions appear to be able to synthesise sufficient vitamin C for optimal functioning of their metabolic processes. Elevated high environmental temperature has been reported to reduce the concentrations of AA in the serum of rats (Squibb *et al.*, 1954), blood (Hunt and Aitken, 1962; Perek and Kendler, 1963; Attia, 1976), plasma and liver of Leghorn hens (Sykes, 1976). Under these heat stress conditions, supplementation of the diet with ascorbic acid, at levels up to 100-200 ppm, has been shown to produce improvements in growth, egg production and eggshell strength, particularly in broilers and in laying hens (Selye, 1950; Thornton and Deeb, 1961; Freeman, 1963; Ahmad *et al.*, 1967; Riker *et al.*, 1967; Lyle and Moreng, 1968; Shannon and Brown, 1969; see Scott, 1976 for review).

Ascorbic acid and vitamin E are perhaps the most studied nutrients, yet their effects are undefined in many aspects. AA and vitamin E not only has its known cofactor roles for several enzymes, but also its biochemical properties. AA and vitamin E may be used as food additives to prevent oxidation as a preservative, or to enhance the nutritive qualities as cofactor roles for several enzymes, AA may also be used to increase acidity as a stabiliser.

High environmental temperatures may bring about a general increase in requirements in terms of milligrams per kilogram of diet for these vitamins required for maintenance of body cellular metabolism simply by the decreasing the total feed consumption.

Thus, as postulated by Selye (1950), animals have the ability to adapt to stresses. Dietary ascorbic acid may have some value in this adaptation, especially when the stress is heat stress, which also decreases the biosynthesis of ascorbic acid. Shannon and Brown (1969) found that hens began to adapt to heat stress after three days, and after 12 days showed no further decline in fasting metabolic rate. Possibly,

a major effect of dietary ascorbic acid is involved in helping hens to adapt to heat stress.

Thus, in conclusion, 1) Dietary vitamin E and AA supplementation partially mitigates the deleterious effects of chronic heat stress upon thyroid hormone economy possibly as a consequence of reduced oxidative damage. 2) Supplementation of the diet with 500mg/kg vitamin C or vitamin E to broilers appears to improve the plasma thyroid hormone levels and to restore function of the somatotrophic/thyrotrophic axis during chronic heat stress without altering the thermoregulatory responses. 3). It is thus proposed that the anti-oxidative vitamin E and AA, a potential anti-stress agent is protective during exposure to high thermal loads and may thus promote growth. 4) This action may mediate the reported improvements of growth rate attributed to vitamin E and AA supplementation in broiler chickens during heat stress, although this awaits confirmation in extensive growth trials.

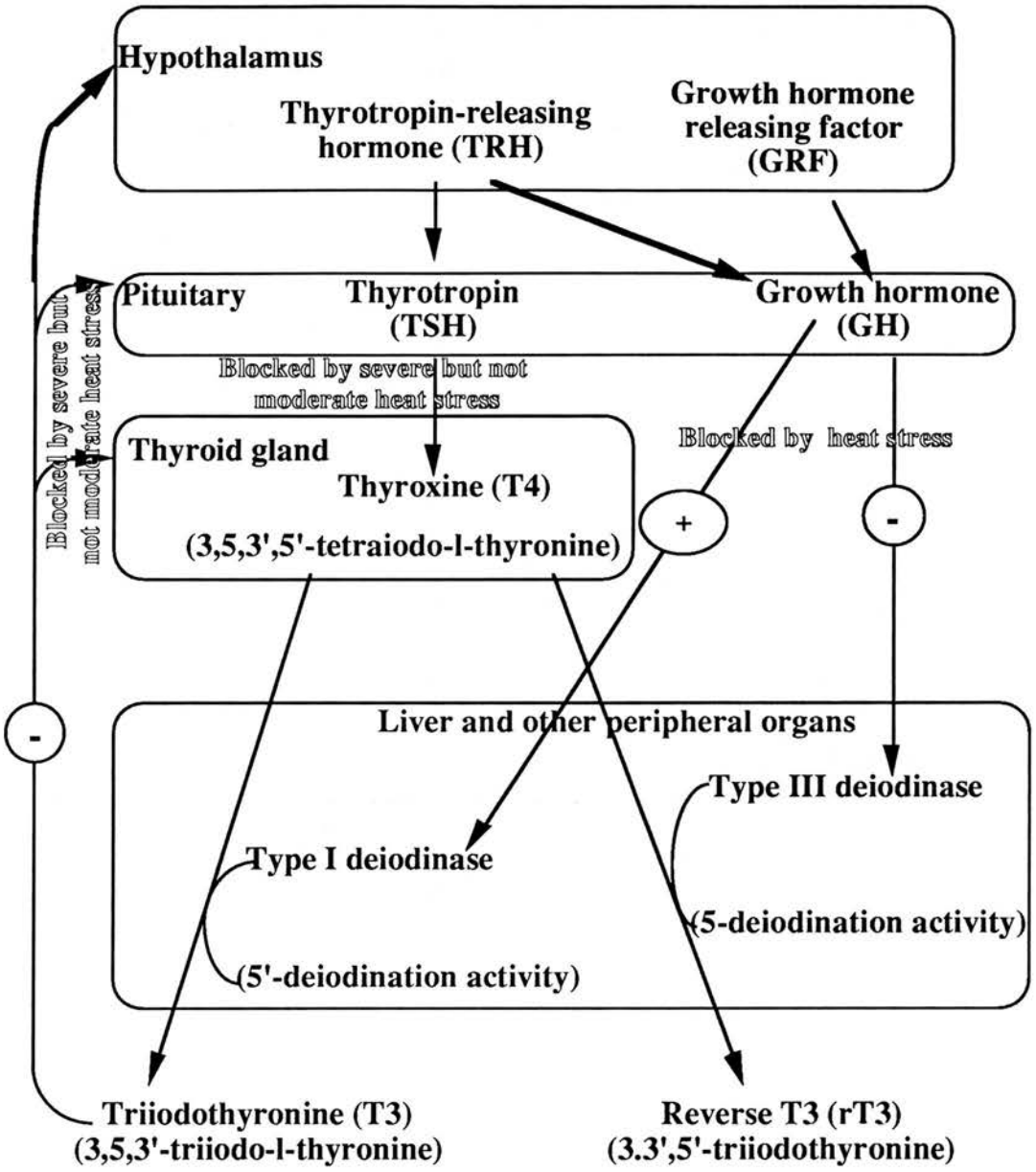
Chapter Eight
General Discussion

8.1. Major contribution of this thesis

The effects of heat stress on the endocrine function of rapidly growing broilers are not well understood. The aim of these series of experiments was to measure the effects of series of different climatic stresses on broiler chickens and to measure the effects of those stresses on various hormonal responses. As a result of the investigation of the responses of plasma thyroid hormones to different "heat loads", we have a much clearer idea of the physiological response of broilers to a variety of different types of heat stress. It was demonstrated that the environmental conditions could be defined by the use of body temperature to measure heat load, which was determined by the complex effects of dry bulb temperature and humidity upon heat exchange. The hormonal response patterns are quite different in moderate and severe heat stress. Moderate heat stress (below 29-31 °C with relative humidity beneath 35 %) may decrease plasma T3 and thus T3 negative feedback control of TSH, and thyroidal T4 secretion, but with no direct inhibitory effect upon the latter. More severe heat stress (above 32 °C or around 29-31 °C with higher humidity) inhibits pituitary and/or thyroid secretion and 5'-monodeiodination (Figure 84). These effects may be related to the extent of hyperthermia induced by a specific heat load but are not the consequence of concomitant decreases in food intake.

Studies of the influence of feed intake on broiler performance at high (35 °C) and thermoneutral (21 °C) ambient temperatures were undertaken, to investigate growth rate and the thyrotrophic mechanisms regulated by GH under these conditions. These studies confirmed that food intake in chickens is inversely related to ambient

Figure 84. Modifications to the hypothalamus-pituitary-thyroid axis and the hypothalamus-pituitary-somatotroph-liver axis in poultry by moderate and severe heat stress.



T3 being derived almost exclusively from the peripheral conversion of T4. This reaction is stimulated by GH when chickens in thermoneutral temperatures environment. GH regulates the peripheral conversion of T4 to T3 by the means of stimulation of the liver type I deiodinase (5'-monodeiodination) activity or inhibition of the liver type III deiodinase (5-monodeiodination) activity when chickens in thermoneutral temperatures environment. The normal functions of the hypothalamus-pituitary-thyroid axis may regulate by the negative feedback of T3.

Chronic heat stress reduces circulating T3, possibly by a reduction in hepatic 5'-monodeiodination. Stimulation of T4 production by negative feedback of peripheral low T3 concentration is blocked by severe but not moderate heat stress condition.

temperature (Prince *et al.*, 1961; Smith and Oliver, 1971) and the decline in growth rate that occurs at this temperature is not entirely the result of reduced feed intake by heat stressed chickens (Fuller and Dale, 1979). The results also support the proposal that reduced food intake is one response of chicken to elevated high temperature (Mitchell and MacLeod, 1983) but that thyroid functions in birds exposed to high temperature are also changed, although reports in the existing literature on the effects of heat stress on thyroid hormones are very confusing (see Figures 8 and 9 in Section 1.7.6.1, page 82-84 and Figure 28 in Section 4.1, following page 140 for details). Furthermore, it was demonstrated that the thyroid hormonal mechanisms regulated by GH in heat stressed broilers and "pair fed" chickens were different (Chapter Three). Although reduced food intake might be linked with changed thyroid function (Mitchell and Raza, 1986a) and the peripheral GH concentration (McMurtry and Johnson, 1988) in fasted birds. Interestingly, although "pair fed" broiler chickens in thermoneutral temperature consumed the same amount of food as heat stressed birds, they grew faster than those maintained at high ambient temperature, and they had normal thyroid functions and normal thyroid hormonal mechanisms regulated by GH (Chapter Three), while heat stressed chickens have reduced plasma concentrations of T3, and T4 despite elevated concentrations of GH (Chapter 3). The results recorded in Chapters 3, 4 and 5 have demonstrated that this phenomenon may be mediated by a reduction in hepatic 5'-monodeiodination and may involve changes in the function and interaction of the thyrotrophic and somatotrophic axes.

In chronic (long term exposed to high ambient temperature), severe and moderate heat stress conditions, stimulation of peripheral T3 production by TRH is inhibited proportionally by heat load despite an increased GH response (Chapters 4 and 5). Chronic, severe and moderate heat stress condition may result in different ways to an altered T4 response to TSH in the thyroid gland and / or TSH response to TRH in the anterior pituitary gland because the T4 response was greatly decreased by

severe heat stress but was unaltered by the moderate treatment. Whilst primarily plasma T4 concentration was reduced in broilers subject to chronic severe heat stress it was increased or unaltered by chronic moderate heat stress during the exposure (Chapters 4 and 5) compared with the control birds (Figure 84).

Thus both moderate and severe heat stress are known to affect hormonal secretion rate and these changes may be the main reason for decreased growth rate at high ambient temperature. There was correlation between changes in T3 and T4 secretion and growth rate in heat stressed chickens, and these two observations may be linked together, but we do not know which is cause and which is effect. Although the exact mechanisms controlling growth rate of heat stressed chickens is still poorly understood, it is not due solely to the influence of feed intake as originally proposed.

Two ways of mitigating these effects were investigated i.e. genetic modification and changes to the diets. There are several major genes which affect the feather structure or the amount of feather covering, and may also affect the response to environmental temperature (see Section 6.3, Page 173 for details). It has been suggested by a number of field trials that broilers carrying the naked neck gene (Na) may be more heat stress resistant, and administration of vitamin C (ascorbic acid) might improve growth rate of commercial broilers under heat stress conditions.

The studies on the effects of humid heat stress condition on chickens with or without naked neck gene (Na) and their response to exogenously administered TRH *in vivo* described in Chapter 6, demonstrated that naked neck birds shifted the response of heat stress in such way as if they were kept at lower temperature than birds of a commercial broiler strain. In the same chronic heat stress conditions (29 °C with 35 or 85 %RH), plasma T4 and T3 levels are higher in naked neck chickens than in modern broilers. Thus different breeds may respond to the same heat load (29 °C with 85%RH) in different ways. Within breeds at the same heat load (29 °C with 85%RH)

when compared with the thermoneutral controls, the plasma T4 levels were increased in naked neck chickens, whereas the plasma T4 levels were decreased in modern broilers. These results suggested that not only the environments should be characterised in terms of their potential influence upon thermal exchange and thermoregulation of bird but also the breeds should be characterised in terms of their feather condition, growth rate and thyroid function. Presently, it is unclear whether the mechanism of heat tolerance in these birds occurs as the consequence of reduced growth rate or due to the expression of the naked neck gene, as both these factors are present in this line.

Since the T4 response was greatly decreased by severe heat stress but was unaltered or increased by the moderate treatment in the modern broiler chickens, the conflicting reports relating to plasma thyroid hormone concentrations in heat stressed poultry may also be due to the use of different diets which may reduce the impairment of heat stress on broilers. It is known that ascorbic acid (AA) can affect the growth rate of broilers at high ambient temperatures but the mechanisms are not known. Therefore ascorbic acid and vitamin E were administrated to broilers under heat stress conditions to see if there were any effects on T3 and T4 production.

The administration of ascorbic acid (AA) to broilers appears to help to maintain the blood pH values in the moderate heat stressed chickens but not in severe heat stressed chickens. Whilst the administration of AA to broilers appears to help to maintain the thyroid hormone production in the severe heat stressed chickens but not in moderate heat stressed ones (Chapter 7). These results suggested that ascorbic acid may use its reducing agent biochemical properties to prevent oxidation as a preservative in the severe heat stress condition and use its chemical acidity properties to reduce alkalinity as a stabiliser in the moderate heat stress condition.

The results of Chapter 7 provided possibly evidence to suggest that the antioxidant properties of both supplemental dietary ascorbic acid and vitamin E may help to maintain the thyroid hormone production in chronic severe heat stress. Supplemental dietary AA may improve the plasma thyroid hormone levels of heat stressed broilers because its reducing agent biochemical properties prevent oxidation of cellular membranes, rather than its acidic properties reducing alkalinity. However, the mechanisms by which AA and vitamin E can improve (increase) the plasma thyroid hormone levels when birds are under stress, still remain unknown.

Thus, in conclusion, the conflicting reports relating to plasma thyroid hormone concentrations in heat stressed poultry may be due to the use of: a) different dry bulb temperatures to induce heat stress; b) different durations of heat stress; c) different heat loads (e.g. humidity) to induce heat stress; d) different ages of birds; e) different strains of birds; f) different diets fed to birds; g) different degrees of thermotolerance.

8.2. Further research projects based on the findings in the Thesis

Collectively, the results in this thesis have demonstrated that the reduction of growth rate in chronic heat stressed chickens is accompanied by a decrease in T3 concentration despite elevated concentrations of GH. Plasma T4 concentration was reduced in broilers subject to chronic severe heat stress but was increased or unaltered by chronic moderate heat stress. This phenomenon may be mediated by a reduction in hepatic 5'-monodeiodination. However, changes in the function and interaction of the thyrotrophic and somatotrophic axes by reducing the number of hepatic GH receptors or GH affinity to hepatic GH receptors which regulate hepatic 5'-monodeiodination, cannot yet be excluded. Further research should, therefore, be focused on the number of hepatic GH receptors or GH affinity to hepatic GH receptors between birds under

different heat load conditions in order to understand the reason why GH fails to stimulate hepatic 5'-monodeiodination in heat stressed birds.

Furthermore, the results in this thesis have demonstrated that the sensitivity of the peripheral 5'-monodeiodination of T4 to T3 in the liver to administration of exogenous TRH, is decreased in normal (commercial) but not in naked neck chickens. This result suggested that the naked neck gene may play an important role in control of GH-receptor binding in liver membranes and thyroid function of chickens exposed to chronic high ambient temperatures. Future research should, therefore, seek for the answer as to why the 5'-monodeiodinase (5'-D) activities in liver cells are different between commercial and naked neck chickens under either heat-stress or non-heat-stress conditions. Better understanding of the reason why the 5'-D activity in liver cells is different between commercial and naked neck chickens might lead breeders to consider incorporating the naked neck gene into a commercial broiler population. Then selection pressure could be placed on multigenic variation in heat resistance, higher thyroid function and large body size. The naked neck gene does not have the disadvantages of the genes which cause removal of all body feathers, and may enable broilers to respond to some hot environment as moderate heat load while commercial modern broilers may respond to the same environment as if it was a severe heat load.

The present studies indicate that providing supplementary vitamin E or AA to broilers appears to increase the plasma thyroid hormone levels in the severe heat stressed chickens. In order to obtain a better understanding of the reason why administration of AA or supplementary vitamin E can improve endocrine function in severe heat stressed chickens, we should investigate the 5'-D activities in liver cells in selenium deficient chickens. Thus loss of glutathione peroxidase activity provides a plausible explanation for many of the consequences of selenium deficiency, especially those that occur in association with a vitamin E deficiency (Arthur, 1991).

In order to have a better understanding of the reason why the impairment of their thyroid hormones (T4, T3) and GH fail to stimulate hepatic 5'-monodeiodination in heat stressed birds, we should also investigate the synthesis of heat shock proteins and their functions because heat shock/stress proteins are synthesized in all cell types under a variety of stressful conditions. It is suggested that they play a physiological role, being involved in the control of gene expression. Some of heat shock proteins (hsp90) is responsible for unfold proteins (reviewed by Georgopoulos *et al.*, 1990; Welch, 1990; Lindquist, 1986). A failure of these mechanisms might not allow the cell to maintain homeostasis and synthesis of normal proteins and folding the proteins to their functional shapes as active enzymes or receptors (Franceschi, 1989).

8.3. Potential application of the findings in the Thesis to poultry industry

In order to improve poultry production in the tropical and subtropical areas, some commercial poultry producers have used cooling water to reduce the ambient temperature in poultry house to prevent irreversible heat prostration occurring. This method may be more efficacious if humidity in the house could also be reduced. A more efficient cooling system to prevent severe heat stress both by reducing dry bulb temperatures and humidities may be able to achieve better poultry productivity for commercial poultry producers.

In order to achieve increased poultry productivity, commercial poultry producers can reduce economic loss when birds are at high ambient temperatures by using supplemental dietary vitamin E or ascorbic acid to reduce the influence of the severe heat stress or by the use of more heat tolerant birds such as those carrying the naked neck gene.

Thus, in conclusion: 1) Selection of broilers for rearing in hot environment could be made for heat tolerance or resistance and improved growth on the basis of thyroid hormone and growth hormone responses to heat stress. 2) Selection on basis of feathering, insulation and naked neck gene may improve heat tolerance or resistance to heat stress. 3) The improvement of thyrotrophic/somatotrophic function by nutritional means e.g. administration of AA and vitamin E may improve heat tolerance or resistance of chicken.

Appendixes

Appendix 1. Data for Section 3.1.3 (figure 19) and Section 3.2.3 (figure 23).
The effect of hot environment and paired feeding in chickens at 21 °C and 35 °C.
Values are expressed as means ± SEM for five female broilers.

Measurement	Thermoneutral						Heat stress		
	Ad libitum at 21 °C			Pair Feeding* at 21 °C			*Ad libitum* at 35 °C		
	Mean	SEM	**	Mean	SEM	**	Mean	SEM	**
Body weight (g)	881	36.224	a	847	43.827	a	703	6.7082	b
Food intake (g/day)	127.6	3.3094	a	90.7	5.4113	b	95.8	4.8746	b
Body weight gain (g/day)	50.7	3.3094	a	41.2	1.118	b	29.9	2.1019	c
FCR***	2.6	0.1342	b	2.4	0.0447	a	3.2	0.0894	c
T4 (ng/ml)	15.15	0.33	a	15.61	2.19	a	5.56	0.49	b
T3 (ng/ml)	3.54	0.28	a	3.03	0.23	a	0.98	0.07	b
GH (ng/ml)	0.65	0.15	a	0.41	0.10	a	1.19	0.27	b

* Paired feeding = broilers who were fed the same amount of food as heat stressed broilers at thermoneutral temperature.
** Values are expressed as means ± SEM for five female broiler chickens. Values within line with different letters are significantly different at P least than 0.05% level.
*** FCR = Feed conversion ratio. Values are expressed as Food Intake:Body weight gain.

Appendix 2. Data for Section 3.1.3 (figure 20) and Section 3.2.3 (figure 22).
The effect of paired feeding at 21 °C and heat stress on broiler chickens release of peripheral hormonal concentration (ng/ml) responses to TRH subcutaneous injections (10 µg/kg body weight).
Values are expressed as means ± SEM for five female broiler chickens.

Hormone	Thermoneutral						Heat stress		
	Ad libitum at 21 °C			Pair Feeding at 21 °C**			*Ad libitum* at 35 °C		
Time*	Mean	SEM	***	Mean	SEM	***	Mean	SEM	***
T4									
0	15.15	0.33	c	15.61	2.19	c	5.56	0.49	a
40	17.60	1.61	cd	22.10	4.66	cd	9.54	0.43	b
80	14.68	1.55	c	9.17	0.38	b	6.69	0.51	a
120	16.59	1.18	c	10.38	0.75	b	6.36	0.63	a
160	9.59	1.17	b	9.51	1.45	ab	8.71	1.03	ab
T3									
0	3.54	0.28	c	3.03	0.23	c	0.98	0.07	a
40	3.20	0.28	c	4.27	0.24	d	1.60	0.19	b
80	4.88	0.22	de	5.32	0.51	e	1.21	0.13	a
120	4.94	0.36	de	4.57	0.22	de	1.00	0.05	a
160	3.41	0.43	c	3.92	0.21	d	1.23	0.10	a
GH									
0	0.65	0.15	ab	0.41	0.10	a	1.19	0.27	c
40	4.75	0.41	d	5.24	1.25	d	10.79	0.40	e
80	1.27	0.28	bc	1.53	0.59	bc	3.39	0.13	d
120	1.09	0.17	bc	1.26	0.33	bc	1.52	0.13	c
160	0.61	0.16	ab	0.65	0.32	abc	1.10	0.23	c

* Time after post subcutaneously injections of TRH are expressed as minute.
** Paired feeding = Broilers who were fed the same amount of food as heat stressed broilers at thermoneutral temperature.
*** Values are expressed as means ± SEM for five female broiler chickens. Values within line with different letters are significantly different at P least than 0.05% level.

Appendix 3. Data for Section 3.3.3 (figure 25). Plasma hormonal concentration (ng/ml) following subcutaneous injections of avian GH (15 µg/kg body weight) to 7-week old chickens. Values are expressed as means ± SEM.

Hormone		Thermoneutral					heat stress		
		Ad libitum at 21 °C			Pair Feeding at 21°C**		*Ad libitum* at 35 °C		
	Time*	Mean	SEM ***		Mean	SEM ***	Mean	SEM ***	
T4									
	0	14.20	2.08	c	17.00	1.13 cd	8.00	0.57	ab
	40	10.70	0.76	bc	16.80	2.27 c	6.20	0.30	a
	80	9.00	0.94	ab	11.00	1.32 b	5.80	0.30	a
	120	9.20	0.91	ab	10.80	0.91 b	6.50	0.23	a
T3									
	0	2.40	0.21	b	2.40	0.11 b	1.00	0.11	a
	40	3.30	0.19	c	3.75	0.23 c	1.15	0.08	a
	80	3.00	0.30	bc	3.90	0.13 c	1.15	0.08	a
	120	2.50	0.23	b	3.10	0.11 c	0.80	0.06	a
GH									
	0	1.34	0.34	a	0.90	0.34 a	1.15	0.34	a
	40	4.80	0.53	cd	4.55	0.77 cd	8.00	0.08	e
	80	1.50	0.42	a	1.60	0.30 a	3.80	0.34	d
	120	1.00	0.08	a	1.70	0.36 b	2.55	0.38	bc

* Time after post subcutaneously injections of TRH are expressed as minute.

** Paired feeding = Broilers who were fed the same amount of food as heat stressed broilers at thermoneutral temperature.

*** Values are expressed as means ± SEM for seven female broiler chickens. Values within line with different letters are significantly different at P least than 0.05% level.

Appendix 4. Data for Section 3.3.3 (figures 26-27). Comparison of body weight (g), body weight gain (g/day), FCR (%) and body temperature (°C) between pair-fed and heat stressed chickens. Values are expressed as means ± SEM, n=7.

Measurement	Thermoneutral						Heat stress		
	Ad libitum at 21 °C			Pair Feeding* at 21 °C			*Ad libitum* at 35 °C		
	Mean	SEM	**	Mean	SEM	**	Mean	SEM	**
Final body weight (g)	2141.3	142.17	a	2067.2	66.484	a	1608.8	70.749	b
Body weight gain (g/d)	65.01	6.192	a	56.51	2.738	b	29.94	2.812	c
Body temperature (°C)	41.137	0.41	a	41.137	0.42	a	42.7	0.39	b
FCR***	1.99	0.038	b	1.67	0.081	a	3.05	0.788	c
Food intake (g/day)	129.69	13.533	a	93.4	3.239	b	91.31	2.983	b

* Paired feeding = Broilers who were fed the same amount of food as heat stressed broilers at thermoneutral temperature.

** Values are expressed as means ± SEM for seven female broiler chickens. Values within line with different letters are significantly different at P least than 0.05% level.

*** FCR = Feed conversion ratio. Values are expressed as:
Food intake (g) : Body weight gain (g).

$$\text{FCR} = \text{Food conversion ratio} = \frac{\text{Food intake (g/d)}}{\text{Body weight gain (g/d)}} = \frac{1}{\text{FCE}}$$

FCE (%, g gain/g intake) = Feed conversion efficiency. Values are expressed as:

$$\text{FCE} = \text{Food conversion efficiency (\%)} = \frac{\text{Body weight gain (g/d) x 100\%}}{\text{Food intake (g/d)}}$$

Appendix 5. Data for Section 4.3 (figure 30). The effects of a range of dry bulb temperatures (relative humidity around 35-45 %RH) upon plasma T4 responses to the injection of TRH in individual trials. Values are expressed as means ± SE.

Trial 1 and 2		Thermoneutral				Heat stress	
		21 °C	45%RH	29 °C	35%RH	35 °C	35%RH
		Mean	SEM	Mean	SEM	Mean	SEM
	Time						
						Peripheral T4 concentration (ng/ml)	
Trial 1	0	16.228	1.5807	17.305	1.5885	8.7130	0.84513
(n=5)	40	23.558	2.1886	23.590	2.3217	11.764	1.5988
	80	12.812	1.0161	10.743	1.9192	7.3590	0.65539
	120	10.730	1.9314	9.6420	1.7534	6.5700	0.53936
	160	10.500	1.4019	9.4380	1.4276	6.7620	0.78541
Trial 2	0	10.460	0.64041	15.320	1.5955	7.2280	0.43683
(n=5)	40	24.580	3.7454	24.345	2.1290	9.2780	1.3734
	80	8.0400	0.31663	17.965	3.0890	7.8680	0.69729
	120	6.6560	0.32960	15.275	2.2420	6.1120	0.42907
Trial 3		Thermoneutral				Heat stress	
(n=5)		21 °C	45%RH	25 °C	45%RH	32 °C	35%RH
		Mean	SEM	Mean	SEM	Mean	SEM
	0	11.632	1.5290	13.302	1.6037	8.2800	1.4248
	40	16.472	0.85999	20.736	0.88548	10.416	1.3161
	80	14.088	0.15921	7.8640	1.7209	3.0620	1.2634
	120	3.3920	0.16860	6.6540	0.83092	2.4580	0.47852
Trial 4		Thermoneutral				Heat stress	
(n=6)		21 °C	45%RH	24 °C	45%RH	29 °C	35%RH
		Mean	SEM	Mean	SEM	Mean	SEM
	0	12.201	1.0941	13.187	0.91407	11.907	1.1182
	40	18.969	1.8088	22.308	0.79527	16.187	1.9498
	80	13.979	0.84022	16.813	0.64217	10.487	1.5183
	120	6.8630	1.2804	13.380	1.1680	8.5820	1.2880

Appendix 6. Data for Section 4.3 (figure 31). The effects of a range of dry bulb temperatures (relative humidity around 35-45 %RH) upon plasma T3 responses to the injection of TRH in individual trials. Values are expressed as means ± SE.

Trial 1 and 2		Thermoneutral		Heat stress			
		21 °C	45%RH	29 °C	35%RH	35 °C	35%RH
		Mean	SEM	Mean	SEM	Mean	SEM
	Time						
						Peripheral T3 concentration (ng/ml)	
Trial 1	0	3.0683	0.13407	2.2467	0.13595	1.6057	0.13818
(n=5)	40	4.0867	0.16657	3.4950	0.33056	2.5229	0.17220
	80	7.2733	0.19090	4.9483	0.41188	3.4343	0.22731
	120	5.7383	0.42425	4.1333	0.40155	3.0071	0.18792
	160	4.8800	0.53325	3.1550	0.28194	2.5829	0.11603
Trial 2	0	3.3620	0.30451	2.0425	0.16035	0.9267	0.20184
(n=5)	40	4.0460	0.18376	2.8425	0.27380	1.3720	0.20568
	80	5.3420	0.071375	4.6400	0.23400	1.6317	0.45732
	120	3.3020	0.31658	3.7575	0.27380	1.2600	0.38861
Trial 3		Thermoneutral				Heat stress	
(n=5)		21 °C	45%RH	25 °C	45%RH	32 °C	35%RH
		Mean	SEM	Mean	SEM	Mean	SEM
	0	3.3980	0.39869	2.9500	0.24149	1.4020	0.28488
	40	3.8960	0.31886	3.7260	0.29118	1.9140	0.25303
	80	4.9620	0.17567	5.6640	0.57968	2.4820	0.61179
	120	3.4040	0.22320	4.6220	0.29056	1.7300	0.32123
Trial 4		Thermoneutral				Heat stress	
(n=6)		21 °C	45%RH	24 °C	45%RH	29 °C	35%RH
		Mean	SEM	Mean	SEM	Mean	SEM
	0	3.0470	0.23164	2.4833	0.082099	1.5733	0.10337
	40	3.4940	0.22854	3.4050	0.10161	1.9183	0.21164
	80	5.5090	0.30307	6.6583	0.19882	3.7783	0.17244
	120	3.9570	0.28147	4.8483	0.13301	2.3883	0.40935

Appendix 7. Data for Section 4.3 (figure 32). The effects of a range of dry bulb temperatures upon plasma T4 and T3 responses to the injection of TRH. Values are pooled from four trials and expressed as means ± SE.

Measurement	Thermoneutral		Moderate heat stress				Severe heat stress			
Room Temperature	21 °C	45%RH	25 °	45%RH	29 °C	35%RH	32 °C	35%RH	35 °C	35%RH
Hormone	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Time (minute)										
T4										
Pre-injection (0)	12.309	0.69667	13.302	1.6037	14.604	0.68435	8.28	1.4248	7.313	0.41521
40	21.285	1.1545	20.736	0.88548	21.788	1.2026	10.416	1.3161	9.204	1.0028
80	12.499	0.64952	7.864	1.7209	14.405	1.207	3.062	1.2634	7.378	0.54739
120	8.697	0.79405	6.654	0.83092	12.257	1.0797	2.458	0.47852	6.005	0.29504
160	9.468	0.71451			11.418	0.85421			5.953	0.65964
T3										
Pre-injection (0)	3.106	0.119	2.95	0.241	1.905	0.072	1.402	0.285	1.119	0.153
40	3.726	0.112	3.726	0.291	2.668	0.15	1.914	0.253	1.781	0.213
80	6.063	0.193	5.664	0.58	4.477	0.154	2.482	0.612	2.171	0.343
120	4.468	0.23	4.622	0.291	3.474	0.185	1.73	0.321	1.848	0.333
160	3.942	0.297			2.41	0.222			2.506	0.137

Appendix 8. Data for Section 4.3 (figure 33). Effects of a range of dry bulb temperatures upon growth performances from 24 to 41 d and body temperatures in female broilers. Values are pooled from four trials and expressed as means ± SE.

Measurement	Thermoneutral			Moderate heat stress				Severe heat stress				
	21 °C	45%RH		25 °	45%RH		29 °C	35%RH		32 °C	35%RH	
	Mean	SEM	*	Mean	SEM	*	Mean	SEM	*	Mean	SEM	*
BWG (g/day)	57.584	3.7505	a	63.167	9.4238	a	49.946	2.0513	b	37.5	4.1105	c
Feed Intake (g/day)	149.72	12.592	a	155.18	22.338	a	107.03	3.9087	c	129.72	12.782	b
FCR (F.Intake/BWG)	2.527	0.1699	b	2.365	0.3225	ab	2.261	0.09203	a	4.108	0.4128	d
Body temperature (°C)	41.38	0.0402	a	41.71	0.059	b	41.94	0.0602	c	42.83	0.1592	d

Measurement	Severe heat stress			
	35 °C	35%RH		
	Mean	SEM	*	
BWG (g/day)	26.933	3.6912	d	
Feed Intake (g/day)	85.33	4.157	d	
FCR (F.Intake/BWG)	3.1432	0.30475	c	
Body temperature (°C)	42.25	0.1657	d	

* Values are expressed as means ± SE. Values within line with different letters (superscripts) are significantly different at P least than 0.05% level.

Appendix 9. Data for Section 5.1.3 (figures 37-38). The effects of different thermal loads upon body weight and body temperature in female broilers. Values are expressed as means ± SE for thirty female broiler chickens in Trial One.

Measurement	Thermoneutral			Heat stress					
	21 °C	45%RH		29 °C	35%RH		29 °C	85%RH	
	Mean	SEM	*	Mean	SEM	*	Mean	SEM	*
Body weight									
20	487.33	15.788	a	467.66	12.752	a	443.09	16.451	a
27	658.91	20.91	b	659.4	17.628	b	624.19	23.706	b
31	855.91	24.839	c	866.16	17.396	c	791.28	25.765	c
38	1237.6	25.725	e	1191.9	23.448	e	1087.7	34.41	d
41	1400.9	39.638	g	1299.6	29.474	f	1214.6	34.372	e
44	1710.3	49.508	i	1569.7	33.195	h	1408.4	27.684	g
Body temperature (°C)									
20	41.49	0.0472	a	41.53	0.0528	a	41.4	0.0574	a
27	41.42	0.0495	a	41.66	0.0466	b	41.7	0.0762	b
31	41.4	0.0414	a	41.86	0.0581	c	41.87	0.0808	bc
38	41.38	0.0402	a	41.94	0.0602	c	42.15	0.0682	d
41	41.37	0.0293	a	41.96	0.0721	c	42.21	0.0879	d

Appendix 10. Data for Section 5.1.3 (figures 39-41). The effects of different thermal loads upon plasma T4, T3 and GH concentrations in female broilers. Values are expressed as means ± SE for six female broiler chickens in Trial One.

Hormone	Thermoneutral			Heat stress					
	21 °C	45%RH		29 °C	35%RH		29 °C	85%RH	
	Mean	SEM	*	Mean	SEM	*	Mean	SEM	*
Age (Days)									
T4							Peripheral T4 concentration (ng/ml)		
22	10.637	1.6118	a	7.71	1.4521	a	8.414	1.259	a
24	11.104	1.1243	a	11.253	2.2307	a	8.37	1.5117	a
27	10.687	1.2876	a	8.741	1.86	a	9.5	1.8914	a
29	9.218	0.57522	a	13.098	1.2856	b	12.736	2.0131	b
31	11.804	1.3591	a	13.741	1.3901	a	12.336	2.1874	a
34	12.711	1.4664	b	15.358	1.099	a	9.41	1.1455	c
36	11.893	2.2323	b	17.158	1.7628	a	10.818	2.0674	b
38	11.354	1.693	b	17.52	1.7391	a	13.584	1.6371	b
41	10.109	0.6136	b	14.839	1.3072	a	5.954	0.43683	e
T3							Peripheral T3 concentration (ng/ml)		
22	4.2005	0.69598	ab	4.067	0.24858	b	4.7747	0.23258	a
24	4.2005	0.69598	a	4.3588	0.5155	a	4.7706	0.70794	a
27	4.3042	0.35383	a	3.1613	0.33734	c	3.3385	0.21482	c
29	4.5259	0.34338	a	3.3238	0.32856	c	2.9597	0.22119	c
31	4.3583	0.41515	a	3.1328	0.2002	c	2.8364	0.27626	c
34	3.7788	0.38833	b	2.8705	0.32701	c	2.0684	0.2018	d
36	4.0217	0.24172	b	2.3403	0.33027	d	1.9338	0.33178	d
38	4.1559	0.30672	b	3.0161	0.54415	d	2.4154	0.25997	d
41	3.0294	0.28181	c	1.9786	0.14477	d	1.1703	0.091938	e
GH							Peripheral GH concentration (ng/ml)		
22	13.987	3.377	b	17.97	2.6246	b	7.262	1.6052	
24	10.433	2.7545	a	9.545	2.0065	a	9.028	1.8302	a
27	7.805	1.882	a	5.172	1.9249	a	7.743	1.8049	a
29	7.062	3.008	a	6.603	1.4383	a	7.299	1.4823	a
31	6.556	1.6677	a	5.672	1.0965	a	4.082	1.0745	a
34	4.943	1.0423	a	2.704	0.49643	a	3.693	0.85283	a
36	5.247	0.98755	a	4.547	0.88672	a	4.138	0.64952	a
38	1.9	0.61196	a	3.108	0.53644	a	2.328	0.37885	a
41	2.924	0.41029	a	2.668	0.42621	a	2.051	0.33721	a

* Values are expressed as means ± SEM for six female broiler chickens. Values within line with different letters (superscripts) are significantly different at P least than 0.05% level.

Appendix 11. Data for Section 5.1.3 (figures 42-43). The effects of different thermal loads upon plasma T3 an T4 concentrations in the second and the third trials. Values are expressed as means ± SE.

Trial 2 (n=5)

	Thermoneutral		Heat stress			
	21 °C	45%RH	29 °C	35%RH	29 °C	85%RH
Hormone	Mean	SEM	Mean	SEM	Mean	SEM
T3					Peripheral T3 concentration (ng/ml)	
	2.70	0.14	1.70	0.09	1.04	0.08
T4					Peripheral T4 concentration (ng/ml)	
	12.77	1.70	13.75	1.42	7.30	0.58

Trial 3 (n=6)

	Thermoneutral		Heat stress			
	21 °C	60%RH	29 °C	50%RH	29 °C	85%RH
Hormone	Mean	SEM	Mean	SEM	Mean	SEM
T3					Peripheral T3 concentration (ng/ml)	
	1.71	0.28	1.15	0.16	0.60	0.06
T4					Peripheral T4 concentration (ng/ml)	
	14.12	2.12	10.07	1.28	3.06	0.74

Appendix 12. Data for Section 5.1.3 (figures 46-48). The effects of different thermal loads upon plasma T4, T3 and GH responses to the injection of TRH. Values are expressed as means ± SE for six female broiler chickens in Trial One.

Hormone	Thermoneutral				Heat stress				
	21 °C	45%RH			29 °C	35%RH		29 °C	85%RH	
	Mean	SEM	*		Mean	SEM	*	Mean	SEM	*
Time										
T4								Peripheral T4 concentration (ng/ml)		
0	10.109	0.6136	b		14.839	1.3072	a	5.954	0.43683	c
40	20.123	1.526	b		24.756	1.8265	a	9.836	1.2035	c
80	13.434	1.5554	a		18.517	2.9063	a	5.714	0.94918	c
120	11.42	1.3799	a		15.183	2.4466	a	5.184	0.79772	c
160	9.469	1.2668	a		12.269	1.5191	a	5.345	0.87733	c
T3								Peripheral T3 concentration (ng/ml)		
0	3.0294	0.28181	a		1.9786	0.14477	b	1.1703	0.091938	c
40	3.4841	0.10921	a		2.5701	0.21592	b	1.7965	0.14207	c
80	6.3783	0.22752	a		5.0249	0.24862	b	3.1134	0.20617	c
120	5.0214	0.2336	a		3.7251	0.13121	b	2.3246	0.20347	c
160	3.8614	0.32272	a		2.0843	0.38392	b	1.7253	0.25426	b
GH								Peripheral GH concentration (ng/ml)		
0	2.924	0.41029	b		2.668	0.42621	a	2.051	0.33721	a
40	10.495	1.4195	b		28.496	1.7338	a	31.724	0.27557	a
80	8.072	1.3558	b		11.993	1.2235	a	14.193	2.5466	a
120	3.916	1.19	b		8.022	3.2623	a	4.816	0.63809	ab
160	2.741	0.39069	b		3.158	0.59359	b	4.016	0.53644	b

* Values are expressed as means ± SEM for six female broiler chickens. Values within line with different letters (superscripts) are significantly different at P least than 0.05% level.

Appendix 13. Data for Section 5.2.3 (figure 49). The effects of different thermal loads upon plasma T3 responses to the injection of TRH in the second, the third and the fourth trials. Values are expressed as means ± SE.

		Thermoneutral		Heat stress			
Trial 2		22 °C	45%RH	29 °C	35%RH	29 °C	85%RH
		Mean	SEM	Mean	SEM	Mean	SEM
	Time						
						Peripheral T3 concentration (ng/ml)	
	0	2.62	0.12	1.85	0.07	0.83	0.09
	40	3.10	0.39	2.64	0.17	0.91	0.10
	80	6.46	0.46	4.30	0.14	1.35	0.16
	120	5.12	0.34	3.34	0.20	1.23	0.14
	160	3.87	0.27	2.30	0.26	0.99	0.15
Trial 3		21 °C	45%RH	29 °C	35%RH	29 °C	85%RH
		Mean	SEM	Mean	SEM	Mean	SEM
	Time						
	0	2.70	0.14	1.70	0.09	1.04	0.08
	40	3.09	0.23	2.55	0.24	1.04	0.09
	80	6.06	0.48	3.96	0.19	1.36	0.28
	120	4.51	0.39	3.46	0.35	1.15	0.21
	160	2.91	0.29	1.91	0.26	0.86	0.10
Trial 4		24 °C	45%RH	29 °C	35%RH	29 °C	85%RH
		Mean	SEM	Mean	SEM	Mean	SEM
	Time						
	0	2.48	0.08	1.57	0.10	1.13	0.03
	40	3.41	0.10	1.92	0.21	1.48	0.09
	80	6.66	0.20	3.78	0.17	2.10	0.19
	120	4.85	0.13	2.39	0.41	1.53	0.29

Appendix 14. Data for Section 5.2.3 (figure 50). The effects of different thermal loads upon plasma T4 responses to the injection of TRH in the second, the third and the fourth trials. Values are expressed as means ± SE.

		Thermoneutral		Heat stress			
Trial 2		22 °C	45%RH	29 °C	35%RH	29 °C	85%RH
		Mean	SEM	Mean	SEM	Mean	SEM
	Time						
						Peripheral T4 concentration (ng/ml)	
	0	13.68	1.72	14.01	0.65	2.80	0.89
	40	19.92	1.18	20.83	1.26	3.67	0.95
	80	14.23	1.21	14.24	1.23	3.35	0.55
	120	10.56	1.63	12.05	1.11	3.11	0.96
	160	11.39	1.65	11.48	0.90	5.04	1.54
Trial 3		21 °C	45%RH	29 °C	35%RH	29 °C	85%RH
		Mean	SEM	Mean	SEM	Mean	SEM
	Time						
	0	12.77	1.70	13.75	1.42	7.30	0.58
	40	21.47	3.30	20.74	3.83	9.17	0.82
	80	13.87	1.77	15.72	2.66	7.75	0.34
	120	10.33	1.15	13.88	3.27	6.22	0.66
	160	8.23	0.94	12.77	1.25	6.57	0.62
Trial 4		24 °C	45%RH	29 °C	35%RH	29 °C	85%RH
		Mean	SEM	Mean	SEM	Mean	SEM
	Time						
	0	13.19	0.91	11.91	1.12	4.00	0.81
	40	22.31	0.80	16.19	1.95	5.64	1.09
	80	16.81	0.64	10.49	1.52	3.07	0.89
	120	13.38	1.17	8.58	1.29	1.94	0.42

Appendix 15. Data for Section 6.1.3 (figures 57-59). Comparison of the physiological parameters between naked neck birds and modern broilers at thermoneutral (22 °C 45% RH) and severe heat load (29 °C 85% RH) condition. Values are expressed as means ± SEM for twelve female broiler chickens.

	*Broilers						**Naked neck birds					
	22 °C 45% RH			29 °C 85% RH			22 °C 45% RH			29 °C 85% RH		
	Mean	SEM	***	Mean	SEM	***	Mean	SEM	***	Mean	SEM	***
Measurement												
Body weight:												
21-day	542.25	23.065	a	540.83	23.487	a	321	9.8554	b	320.08	6.5385	b
44-day	1802.2	48.826	a	1590.2	24.168	b	935.3	33.844	c	951.9	17.033	c
Body temperature:												
21-day	41.74	0.0312	a	41.85	0.0892	a	41.425	0.1094	b	41.375	0.0462	b
44-day	41.5	0.1315	a	42.17	0.1764	b	41.683	0.1012	a	41.544	0.0943	a
BWG	55.14	1.526	a	46.33	1.7506	b	27.478	1.0586	c	27.928	0.6007	c

* Broilers = The modern female broiler chickens without naked neck gene (Br).
** NN birds = The 10-year randombred line of female broiler chickens with the incompletely dominant naked neck gene (Na).
*** Values are expressed as means ± SEM for twelve female broiler chickens. Values within line with different letters (superscripts) are significantly different at P least than 0.05% level.

Appendix 16. Data for Section 6.1.3 (figures 60-63). Comparison of peripheral T4 and T3 concentrations (ng/ml) responses to TRH injections (10 µg/kg body weight) between naked neck (NN) birds and modern broilers at thermoneutral (22 °C 45% RH) and severe heat load (29 °C 85% RH) condition. Values are expressed as means ± SEM for five female broiler chickens.

Hormone	*Broilers						**Naked neck birds					
	Thermoneutral 22 °C 45% RH			Heat stress 29 °C 85% RH			Thermoneutral 22 °C 45% RH			Heat stress 29 °C 85% RH		
	Mean	SEM	***	Mean	SEM	***	Mean	SEM	***	Mean	SEM	***
Time												
T4												
0	13.675	1.72	a	2.801	0.89219	d	7.046	0.55723	c	10.53	1.9114	a
40	19.917	1.1811	a	3.668	0.95346	d	10.406	1.1735	b	16.76	2.3519	a
80	14.234	1.2128	a	3.345	0.54828	d	6.247	0.71688	b	12.126	1.1064	a
120	10.564	1.6332	a	3.108	0.95614	d	4.606	0.66903	c	9.792	1.2325	a
160	11.389	1.6507	a	5.04	1.5384	d	5.14	0.72046	d	11.748	2.9069	a
T3												
0	2.6162	0.12021	b	0.8253	0.08891	d	3.2769	0.11382	a	1.2608	0.10094	c
40	3.0994	0.39279	a	0.9106	0.1017	d	3.3949	0.2042	a	1.6307	0.27356	c
80	6.4596	0.46036	a	1.3546	0.16471	d	4.7543	0.1728	b	3.9844	0.30692	c
120	5.1193	0.33783	a	1.2349	0.14432	d	3.3692	0.18385	b	2.9985	0.14141	b
160	3.8739	0.2736	a	0.9885	0.14727	d	2.6295	0.08457	b	1.9828	0.29547	c

* Broilers = The modern female broiler chickens without naked neck gene (Br).
** NN birds = The 10-year randombred line of female broiler chickens with the incompletely dominant naked neck gene (Na).
*** Values are expressed as means ± SEM for five female broiler chickens. Values within line with different letters (superscripts) are significantly different at P least than 0.05% level.

Appendix 17. Data for Section 6.2.3 (figures 66 and 68). The effects of different thermal loads upon plasma T4 responses to the injection of TRH between naked neck (NN) birds and modern broilers. Values are expressed as means ± SEM for six female broiler chickens.

Peripheral T4 concentration (ng/ml)

Breeds	Thermoneutral			Heat stress					
	24 °C	45%RH		29 °C	35%RH		29 °C	85%RH	
Time	Mean	SEM	*	Mean	SEM	*	Mean	SEM	*
Broilers**									
0	13.187	0.91407	a	11.907	1.1182	a	3.996	0.8116	c
40	22.308	0.79527	a	16.187	1.9498	b	5.64	1.09	c
80	16.813	0.64217	a	10.487	1.5183	b	3.072	0.88998	d
120	13.38	1.168	a	8.582	1.288	b	1.938	0.41968	d
Naked neck birds***									
0	8.05	0.64095	c	12.865	1.2076	a	12.67	1.1296	a
40	15.35	1.1619	b	17.397	1.5281	b	18.735	1.7432	b
80	11.255	1.704	b	15.047	3.1778	a	10.288	1.0553	b
120	8.592	1.0476	b	14.233	4.2662	a	8.797	1.4354	b

* Values are expressed as means ± SEM for six female broiler chickens. Values within line with different letters (superscripts) are significantly different at P least than 0.05% level.
** Broilers = The modern female broiler chickens without naked neck gene (Br).
*** NN birds = The 10-year randombred line of female broiler chickens with the incompletely dominant naked neck gene (Na).

Appendix 18. Data for Section 6.2.3 (figures 67 and 69). The effects of different thermal loads upon plasma T3 responses to the injection of TRH between naked neck (NN) birds and modern broilers. Values are expressed as means ± SEM for six female broiler chickens.

Peripheral T3 concentration (ng/ml)

Breeds	Thermoneutral			Heat stress					
	24 °C	45%RH		29 °C	35%RH		29 °C	85%RH	
Time	Mean	SEM	*	Mean	SEM	*	Mean	SEM	*
Broilers**									
0	2.4833	0.082099	b	1.5733	0.10337	c	1.13	0.025965	d
40	3.405	0.10161	a	1.9183	0.21164	c	1.4783	0.094101	d
80	6.6583	0.19882	a	3.7783	0.17244	d	2.0983	0.18567	e
120	4.8483	0.13301	a	2.3883	0.40935	d	1.5267	0.29471	e
Naked neck birds***									
0	3.3933	0.15513	a	2.4667	0.040947	b	1.7617	0.16979	c
40	3.65	0.14648	a	2.9667	0.17661	b	2.0767	0.13893	c
80	5.885	0.28202	b	5.0133	0.13174	c	4.0317	0.22772	d
120	5.0267	0.38914	a	4.1683	0.15628	b	3.365	0.62258	c

* Values are expressed as means ± SEM for six female broiler chickens. Values within line with different letters (superscripts) are significantly different at P least than 0.05% level.
** Broilers = The modern female broiler chickens without naked neck gene (Br).
*** NN birds = The 10-year randombred line of female broiler chickens with the incompletely dominant naked neck gene (Na).

Appendix 19. Data for Section 6.2.3 (figures 65 and 70). The effects of different thermal loads upon food intake (FI), body weight gain (BWG), feed conversion ratio (FCR) and body temperature (Tb) between naked neck chickens and modern broilers. Values are expressed as means ± SEM for six female chickens.

					Peripheral T3 concentration (ng/ml)	
Breeds	**Thermoneutral**		**Heat stress**			
	24 °C	45%RH	29 °C	35%RH	29 °C	85%RH
Time	Mean	SEM	Mean	SEM	Mean	SEM
Broilers**						
FI (g/day)	112.0	5.8	117.4	4.6	93.7	5.0
BWG (g/day)	57.1	8.1	57.0	2.5	40.4	1.1
FCR	2.1	0.2	2.1	0.0	2.3	0.1
Tb (°C)	41.61	0.111	41.93	0.101	42.27	0.131
Naked neck birds***						
FI (g/day)	79.6	8.1	74.0	2.5	74.9	3.5
BWG (g/day)	32.8	0.7	32.6	5.4	30.5	1.5
FCR	2.4	0.2	2.3	0.2	2.5	0.3
Tb (°C)	41.53	0.039	41.52	0.046	41.61	0.034

Appendix 20. Data for Section 7.1.3 (figures 72-76). The effects of administration of ascorbic acid (vitamin C) upon plasma T3 concentration in modern broiler chickens exposed to different humid hyperthermal load conditions. Values are expressed as means ± SEM for six female broiler chickens.

		Thermoneutral (24 °C 45%RH)				Moderate heat stress (29 °C 35%RH)				Severe heat stress (29 °C 85%RH)			
		Control		Vitamin C		Control		Vitamin C		Control		Vitamin C	
Measurement	Age (day)	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
FI (g/day)		121.25	2.64	117.4	8.47	104.42	6.197	108.21	4.029	85.9	5.65	84.7	26.167
BWG (g/day)		52.26	23.271	54.1	2.627	43.5	3.989	51	20.772	38.33	4.31	35.6	4.771
FCR (FI/BWG)		2.32	1.941	2.17	0.16	2.40	0.23	2.12	1.138	2.54	0.409	2.38	0.936
T4	41	11.008	1.1092	11.467	0.6401	11.987	1.633	13.595	1.7306	6.697	1.3713	8.355	1.4766
T3	41	2.4438	0.116	2.4853	0.1563	2.137	0.23156	2.0941	0.1489	1.1236	0.21111	1.817	0.06573
pH	41	7.26	0.027	7.27	0.025	7.33	0.015	7.24	0.016	7.29	0.016	7.27	0.022
BT	41	41.9	0.191	41.74	0.068	42.2	0.045	42.18	0.189	42.53	0.196	42.46	0.202
Body temperature (°C)													
	20	41.53	0.025	41.46	0.129	41.7	0.086	41.8	0.132	41.53	0.034	41.6	0.095
	22	41.74	0.202	41.69	0.068	42.02	0.056	41.82	0.066	42.24	0.096	42.1	0.116
	24	41.71	0.205	41.68	0.07	41.98	0.071	41.73	0.052	42.17	0.124	42.01	0.103
	27	41.89	0.238	41.87	0.092	42.12	0.091	41.81	0.078	42.27	0.156	42.08	0.102
	29	41.7	0.228	41.68	0.086	41.92	0.091	41.62	0.07	42.07	0.156	41.88	0.102
	31	41.6	0.249	41.62	0.049	41.87	0.071	41.7	0.036	42.03	0.147	41.9	0.1
	34	41.45	0.095	41.36	0.081	41.82	0.06	41.6	0.086	42.1	0.068	41.96	0.175
	36	41.76	0.196	41.69	0.065	42.05	0.048	41.87	0.072	42.28	0.087	42.15	0.123
	38	41.67	0.144	41.55	0.072	42.01	0.03	41.89	0.115	42.32	0.104	42.21	0.17
	41	41.9	0.191	41.74	0.068	42.2	0.045	42.18	0.189	42.53	0.196	42.46	0.202

	24 °C 45%RH		29 °C 35%RH		29 °C 85%RH	
	Control	Vitamin C	Control	Vitamin C	Control	Vitamin C
Measurement	%	%	%	%	%	%
FI (g/day)	100 a	97 a	86 b	89 b	71 c	70 c
BWG (g/day)	100 abc	104 a	83 b	98 abc	73 c	68 c
FCR (FI/BWG)	100 ab	94 a	103 ab	91 ab	109 b	103 ab

Appendix 21. Data for Section 7.2.3 (figure 79). The effects of administration of vitamin E upon growth performances in broiler chickens exposed to different heat load conditions. Values are expressed as means ± SEM for six female broiler chickens.

Measurement	Thermoneutral (21 °C 60%RH)				Moderate heat stress (29 °C 50%RH)				Severe heat stress (29 °C 85%RH)			
	Control		Vitamin E		Control		Vitamin E		Control		Vitamin E	
Age (day)	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Food intake (g/period)												
37-44	490.17	15.6727	496.5	20.4083	442.43	26.8464	456.17	27.4996	430.83	24.8174	428.29	27.6956
44-51	790.5	55.3625	691.33	38.3917	660.14	53.6275	594.5	25.7972	726.67	27.1444	600.86	93.7052
51-58	606.17	25.6829	646.33	39.7756	571.29	34.0357	556.67	35.9953	510.67	39.1959	420.33	92.2315
Body weight gain (g/period)												
37-44	326.5	25.438	325.17	15.2562	292.14	11.7943	305.17	25.3032	246	27.3036	127.29	76.3833
44-51	223.67	6.9566	252.33	19.8654	195.29	13.7416	197.33	47.6017	183.33	23.8989	112	70.4922
51-58	171.83	36.4076	274.83	27.124	205.83	16.9627	221.5	23.1436	144.67	37.3588	172.4	68.304
FCE (Food intake/Body weight gain)												
37-44	1.828	0.0551	1.877	0.1686	2.261	0.178	1.993	0.1045	2.005	0.1	2.192	0.2033
44-51	2.473	0.1862	2.162	0.189	2.255	0.1425	2.337	0.1662	3.035	0.2331	2.428	0.8247
51-58	2.73	0.1662	2.588	0.1188	2.996	0.2903	4.415	1.7138	2.891	0.2331	2.384	0.4956

Appendix 22. Data for Section 7.2.3 (figure 80). The effects of administration of vitamin E upon plasma T4 and T3 concentration in broiler chickens exposed to different heat load conditions. Values are expressed as means ± SEM for six female broiler chickens.

Hormones	Thermoneutral (21 °C 60%RH)					Moderate heat stress (29 °C 50%RH)					Severe heat stress (29 °C 85%RH)				
	Control			Vitamin E		Control			Vitamin E		Control			Vitamin E	
	Mean	SEM	*	Mean	SEM *	Mean	SEM	*	Mean	SEM *	Mean	SEM	*	Mean	SEM *
T4	14.123	2.1155	a	12.422	0.9622 ab	10.068	1.2778	b	10.493	1.1455 b	3.062	0.74301	d	6.487	1.5379 c
T3	1.7133	0.2822	a	1.855	0.2315 a	1.15	0.15673	b	1.255	0.0884 b	0.5967	0.06369	c	1.187	0.15918 b

* Values are expressed as means ± SEM for six female broiler chickens. Values within line with different letters (superscripts) are significantly different at P least than 0.05% level.

Appendix 23. Data for Section 7.2.3 (figure 81). The effects of administration of vitamin E upon blood pH value and body temperature in broiler chickens exposed to different heat load conditions. Values are expressed as means ± SEM for six female broiler chickens.

	Thermoneutral (21 °C 60%RH)				Moderate heat stress (29 °C 50%RH)				Severe heat stress (29 °C 85%RH)			
	Control		Vitamin E		Control		Vitamin E		Control		Vitamin E	
Measurement	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Age (day)												
Blood pH value												
3 7	7.282	0.0196	7.268	0.0159	7.281	0.0229	7.315	0.0122	7.285	0.0249	7.286	0.0147
4 4	7.248	0.0122	7.223	0.0237	7.249	0.0176	7.268	0.0131	7.267	0.02	7.271	0.0196
5 1	7.237	0.0282	7.23	0.0314	7.256	0.0245	7.283	0.0065	7.288	0.0131	7.278	0.0265
58	7.255	0.0184	7.213	0.0294	7.286	0.0261	7.293	0.0086	7.303	0.0139	7.296	0.0184
Body temperature (°C)												
3 7	41.2	0.0576	41.167	0.1404	41.614	0.1139	41.583	0.1221	41.95	0.1523	42.043	0.2013
4 4	41.8	0.0816	41.783	0.0543	42.129	0.1911	42.283	0.1723	42.4	0.238	42.543	0.1996
5 1	41.5	0.0633	41.453	0.0559	42.329	0.1196	42.133	0.1743	42.433	0.1943	42.333	0.2972
58	41.35	0.0621	41.417	0.06	42.186	0.1139	42.283	0.2041	42.5	0.1212	42.34	0.305

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